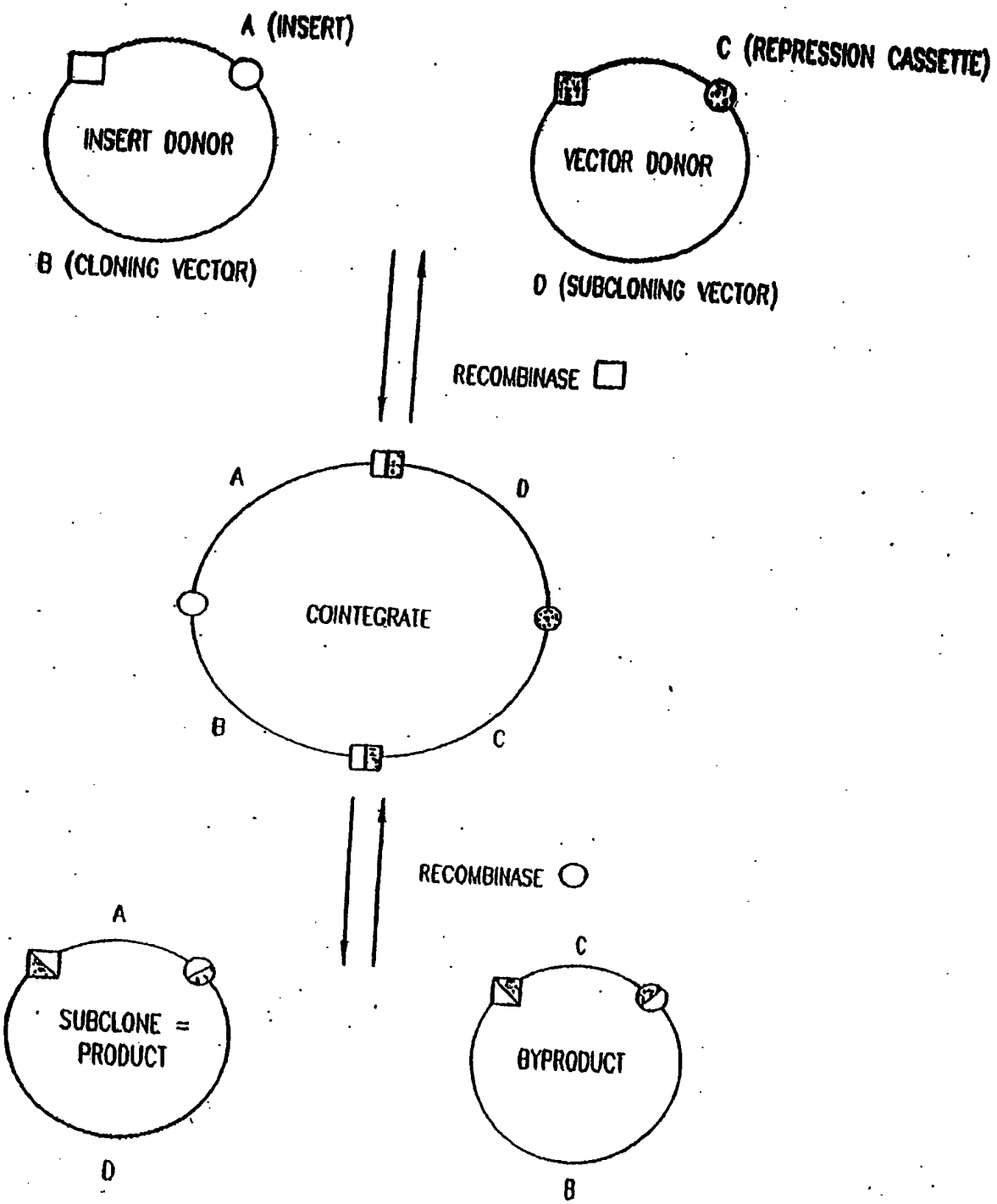

A (INSERT)
INSERT DONOR
B (CLONING VECTOR)
C (REPRESSION CASSETTE)
VECTOR DONOR
D (SUBCLONING VECTOR)
RECOMBINASE
A
D
COINTEGRATE
B
C
RECOMBINASE
A
SUBCLONE = PRODUCT
D
C
BYPRODUCT
B

FIG. 1

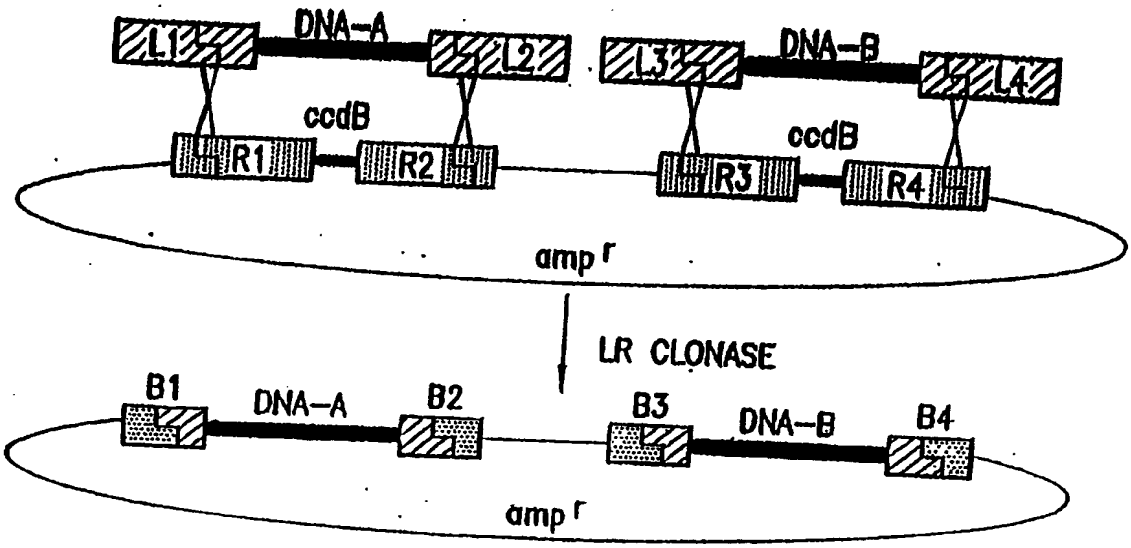

L1
DNA–A
L2
L3
DNA–B
L4
ccdB
R1
R2
ccdB
R3
R4
amp r
LR CLONASE
B1
DNA–A
B2
B3
DNA–B
B4
amp r

FIG. 2

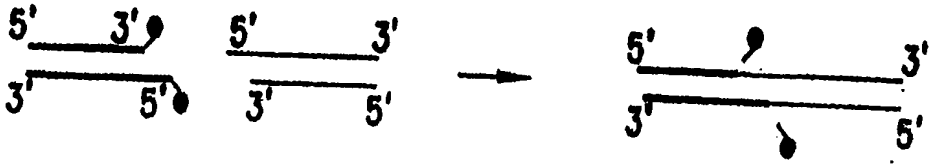


FIG. 3A

FIG. 3B

FIG. 3C

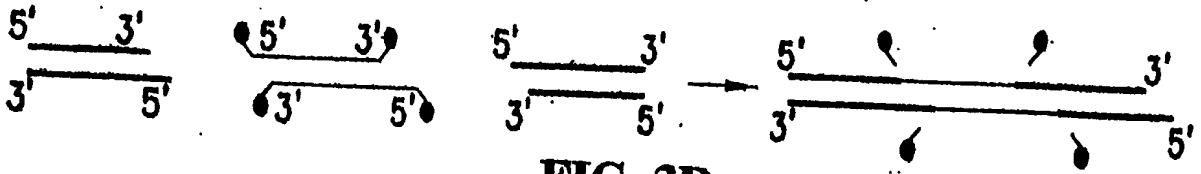


FIG. 3D

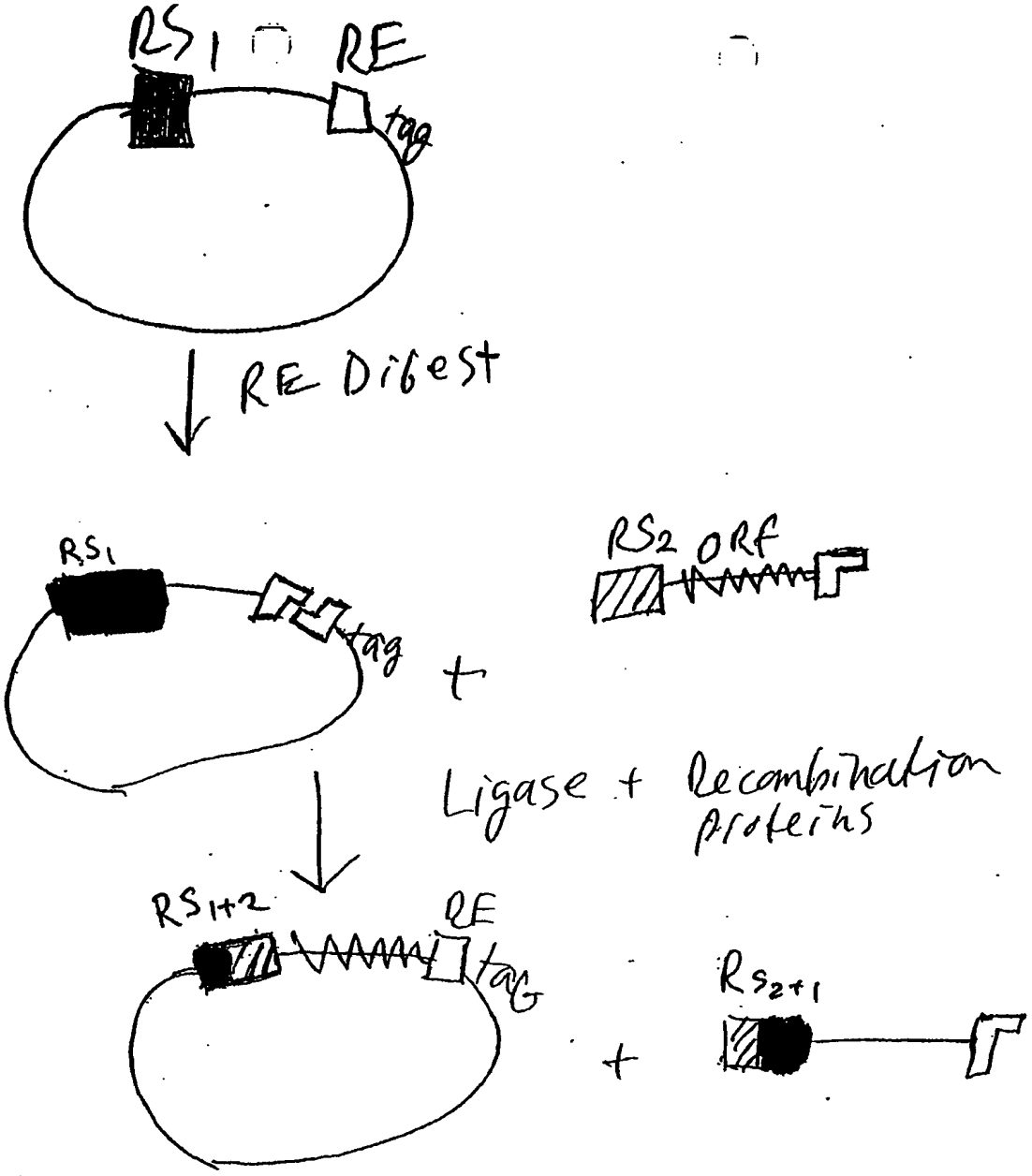

RS1
RE
tag
RE Digest
RS1
tag
+
RS2 ORF
Ligase + Recombination proteins
RS1+2
RE
tag
+
RS2+1

Fig. 4

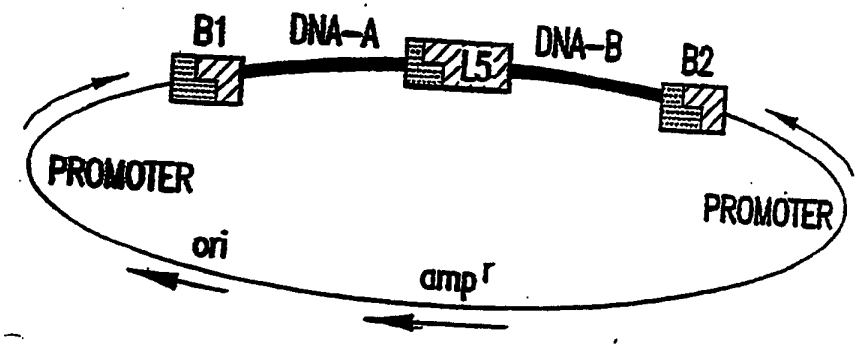


FIG. 5A

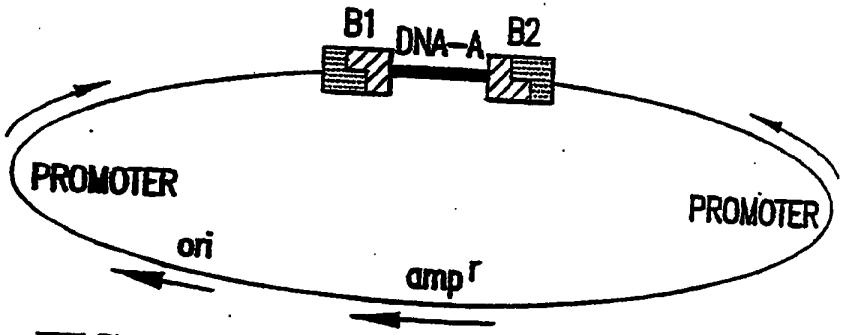


FIG. 5B

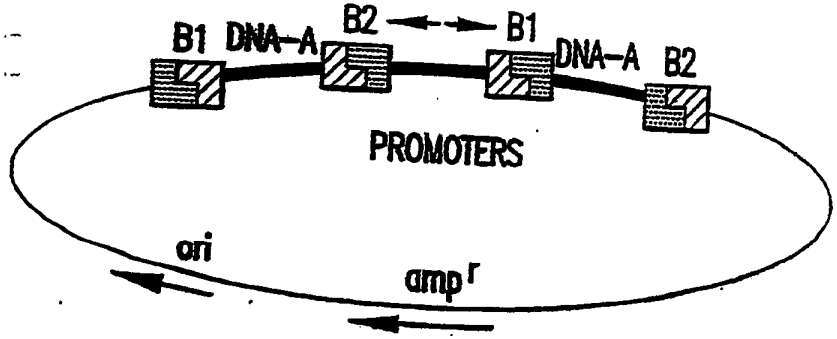


FIG. 5C

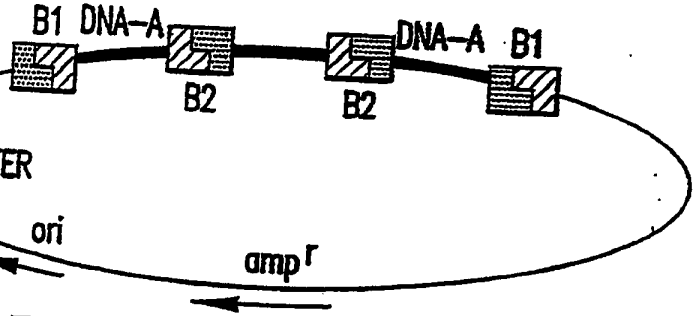


FIG. 5D

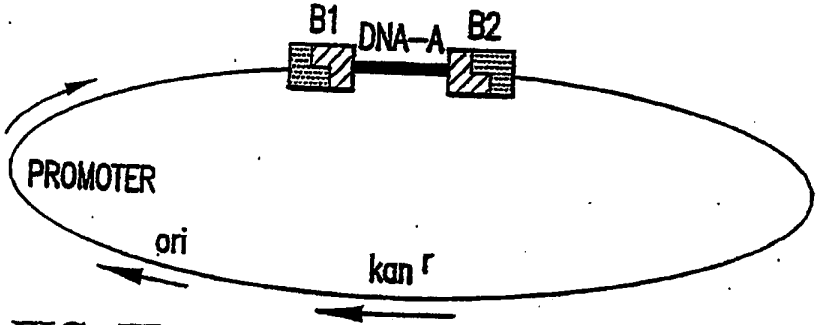


FIG. 5E

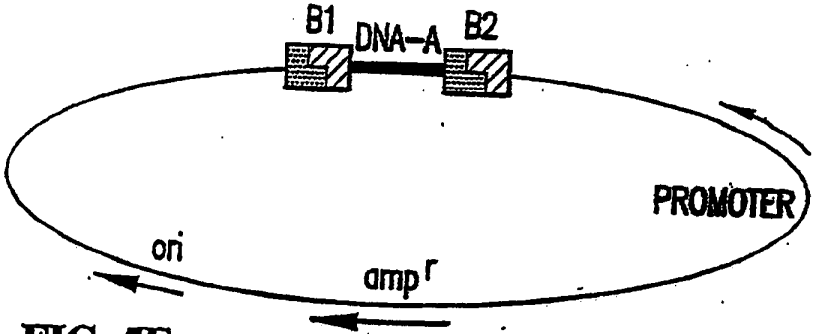


FIG. 5F

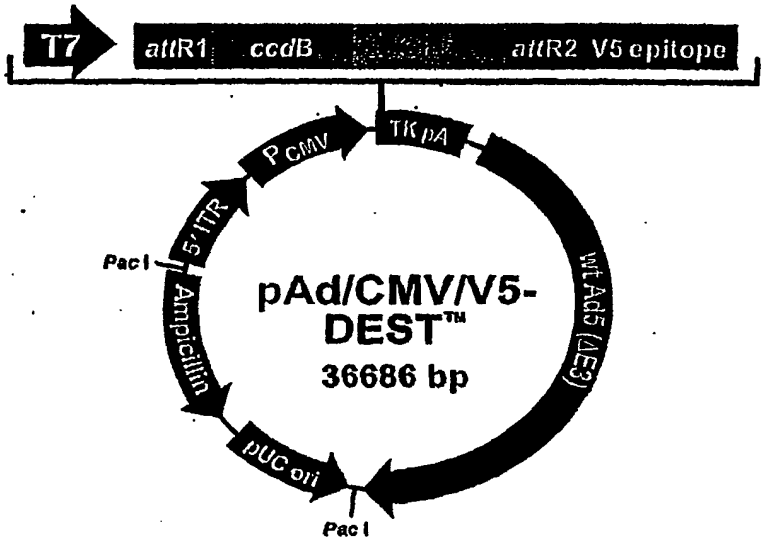

T7
attR1
ccdB
attR2
V5 epitope
P CMV
TK pA
5' ITR
Pac I
pAd/CMV/V5-DEST™
36686 bp
wt Ad5 (ΔE3)
Ampicillin
pUC ori
Pac I

FIG. 6

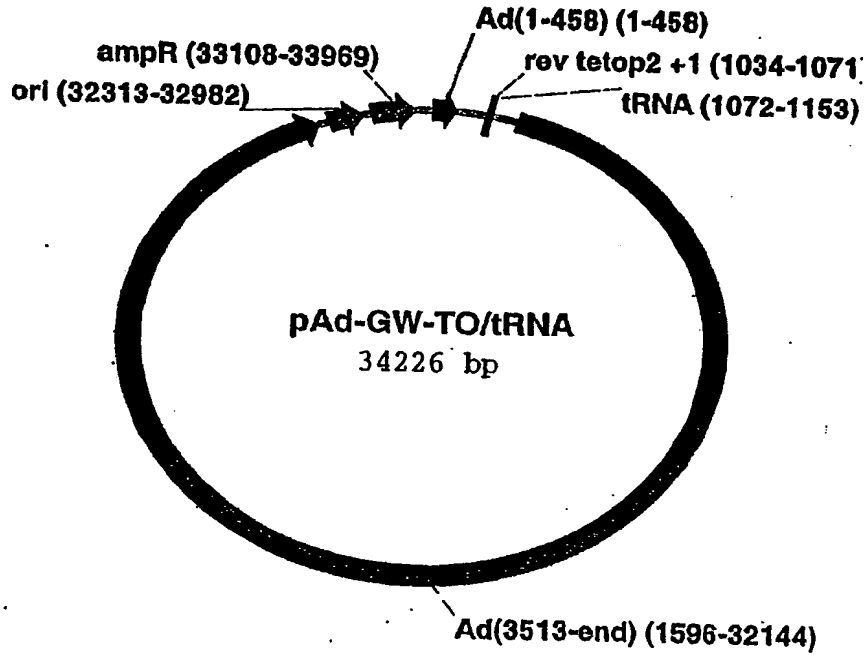

Ad(1-458) (1-458)
ampR (33108-33969)
rev tetop2 +1 (1034-1071
ori (32313-32982)
tRNA (1072-1153)
pAd-GW-TO/tRNA
34226 bp
Ad(3513-end) (1596-32144)

FIG. 7

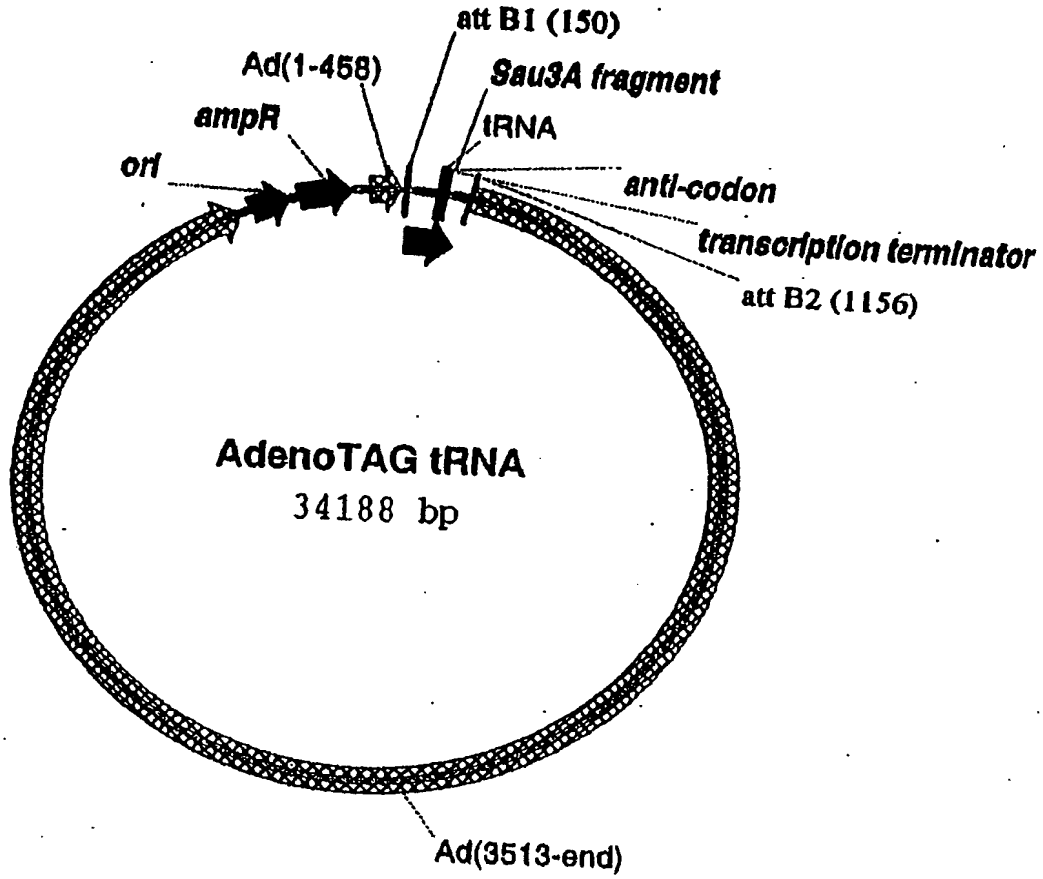

att B1 (150)
Ad(1-458)
Sau3A fragment
ampR
tRNA
ori
anti-codon
transcription terminator
att B2 (1156)
AdenoTAG tRNA
34188 bp
Ad(3513-end)

FIG. 8

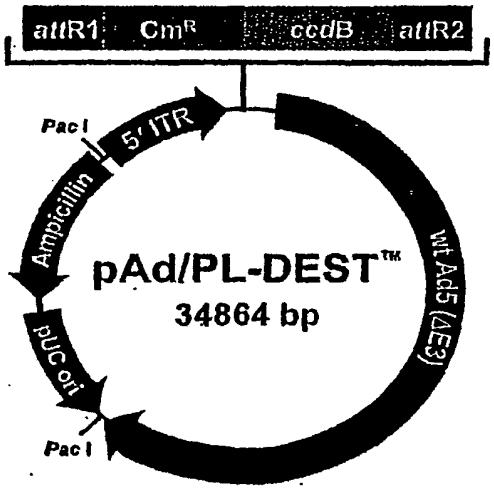

attR1
Cm^R
ccdB
attR2
Pac I
5' ITR
Ampicillin
pAd/PL-DEST™
34864 bp
pUC ori
Pac I
wt Ad5 (ΔE3)

FIG. 9

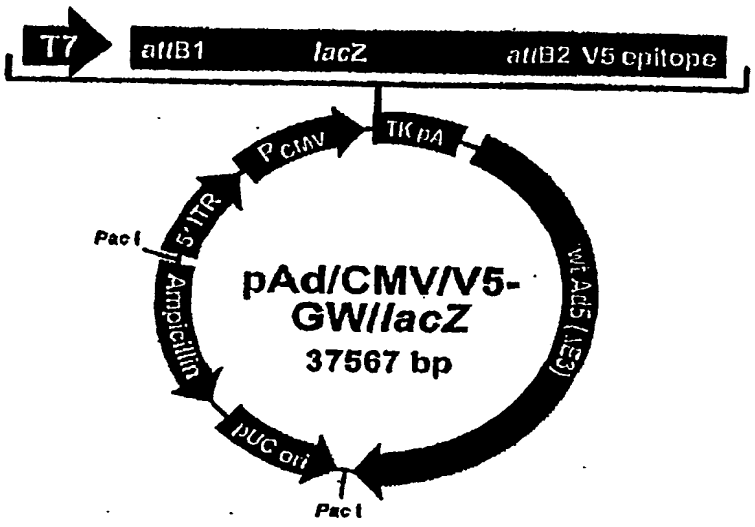

T7
attB1
lacZ
attB2 V5 epitope
P CMV
TK pA
5' ITR
Pac I
pAd/CMV/V5-GW/lacZ
37567 bp
wt Ad5 (Ψ3)
Ampicillin
pUC ori
Pac I

FIG. 10

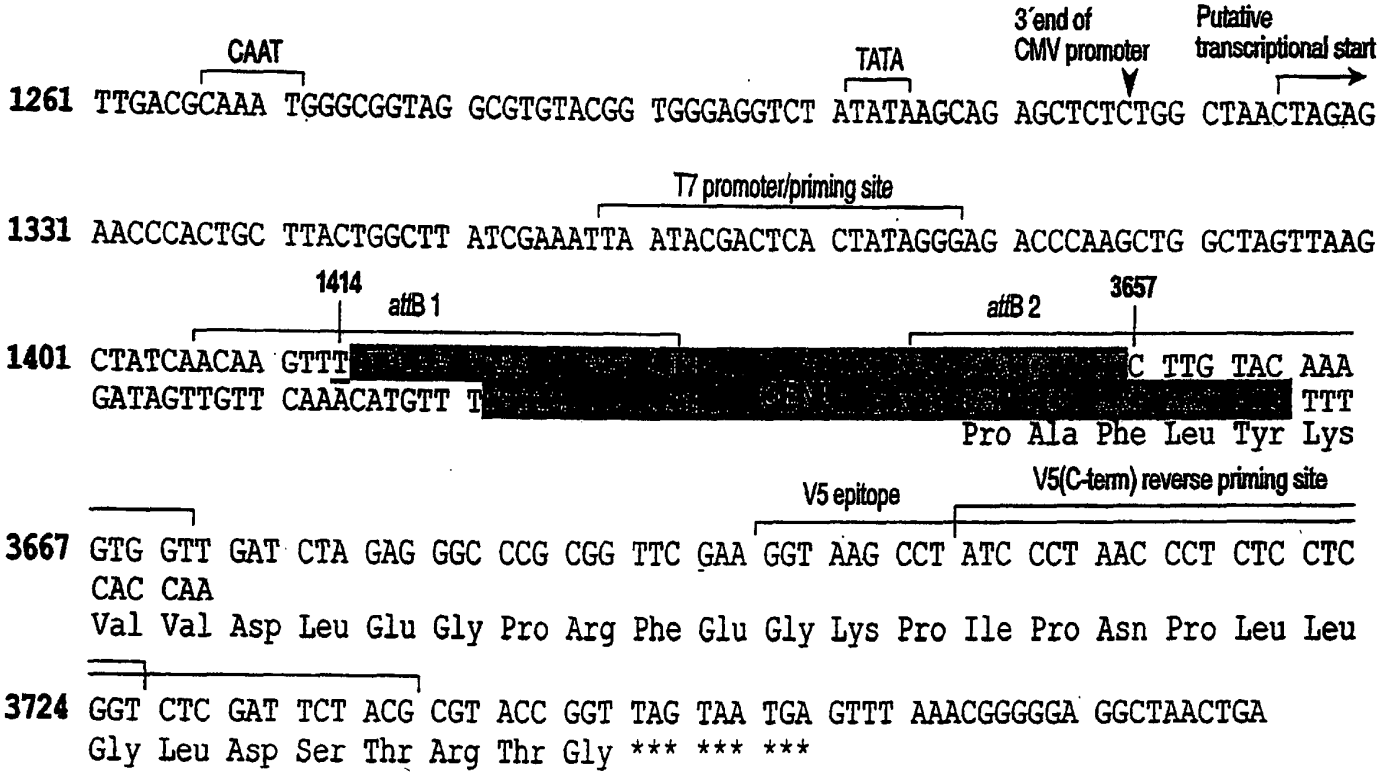

CAAT
TATA
3´end of CMV promoter
Putative transcriptional start
1261 TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAACTAGAG
T7 promoter/priming site
1331 AACCCACTGC TTACTGGCTT ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTG GCTAGTTAAG
1414
attB 1
attB 2
3657
1401 CTATCAACAA GTTT C TTG TAC AAA
GATAGTTGTT CAAACATGTT T TTT
Pro Ala Phe Leu Tyr Lys
V5 epitope
V5(C-term) reverse priming site
3667 GTG GTT GAT CTA GAG GGC CCG CGG TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC
CAC CAA
Val Val Asp Leu Glu Gly Pro Arg Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu
3724 GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTT AAACGGGGGA GGCTAACTGA
Gly Leu Asp Ser Thr Arg Thr Gly *** *** ***

FIG. 11

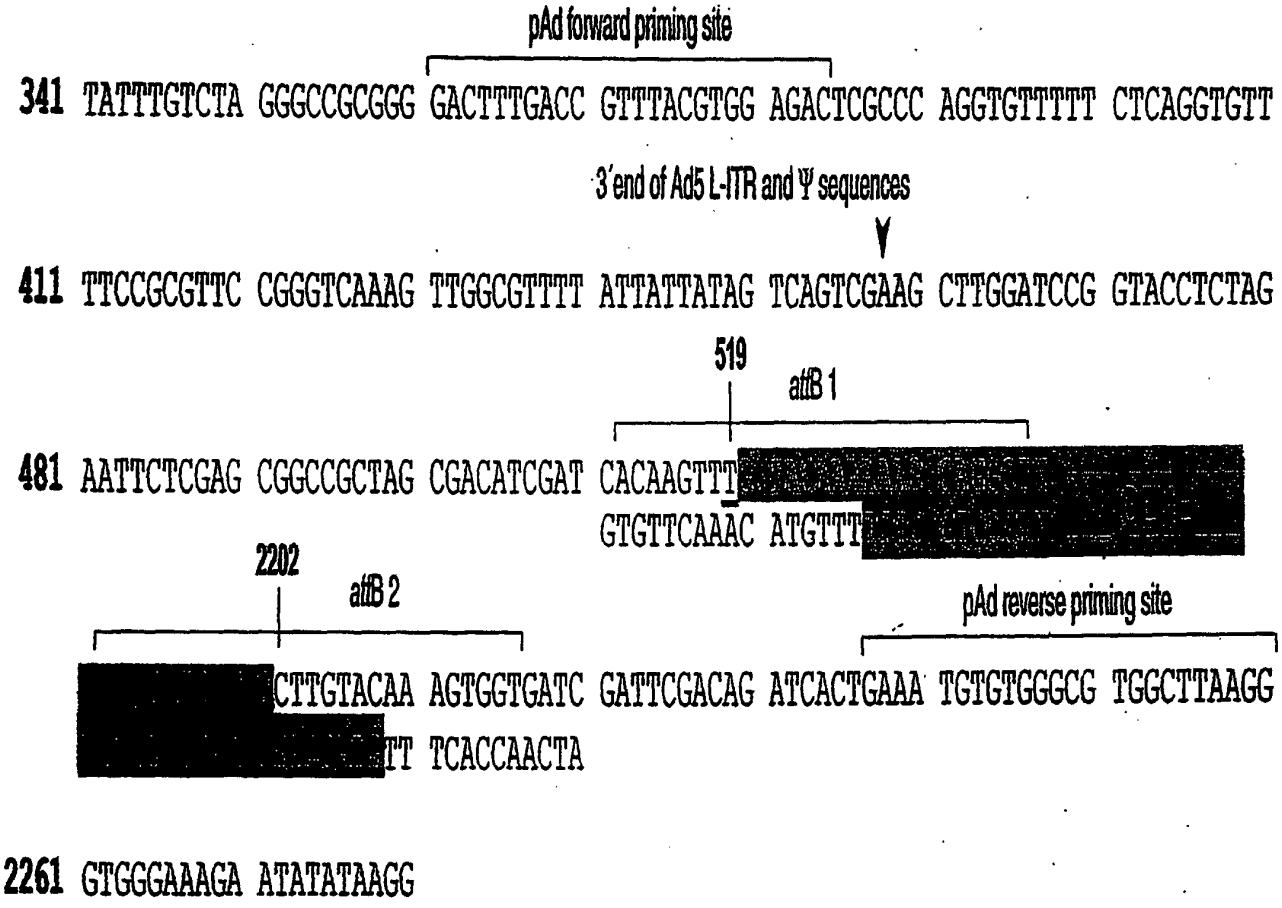

pAd forward priming site
341 TATTTGTCTA GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT CTCAGGTGTT
3'end of Ad5 L-ITR and Ψ sequences
411 TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG TCAGTCGAAG CTTGGATCCG GTACCTCTAG
519
attB 1
481 AATTCTCGAG CGGCCGCTAG CGACATCGAT CACAAGTTT
GTGTTCAAAC ATGTTT
2202
attB 2
pAd reverse priming site
CTTGTACAA AGTGGTGATC GATTCGACAG ATCACTGAAA TGTGTGGGCG TGGCTTAAGG
TT TCACCAACTA
2261 GTGGGAAAGA ATATATAAGG

FIG. 12

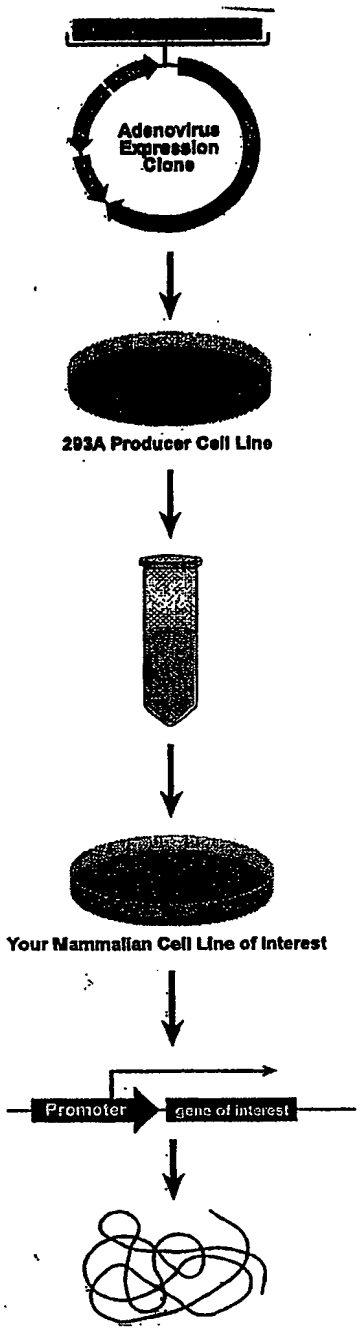

Adenovirus Expression Clone
293A Producer Cell Line
Your Mammalian Cell Line of Interest
Promoter
gene of interest

FIG. 13

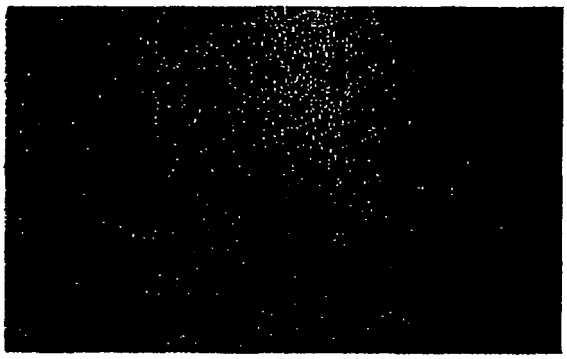


FIG. 14A

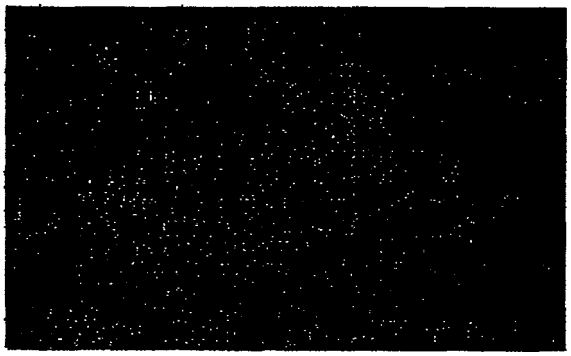


FIG. 14B

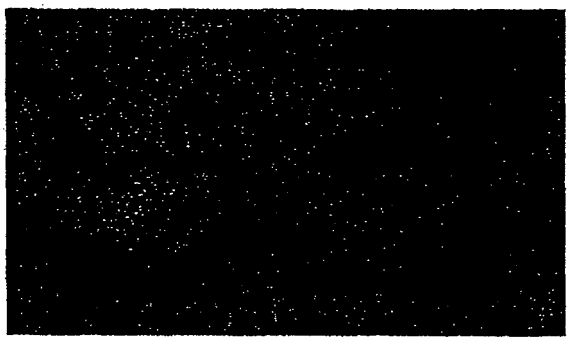


FIG. 14C

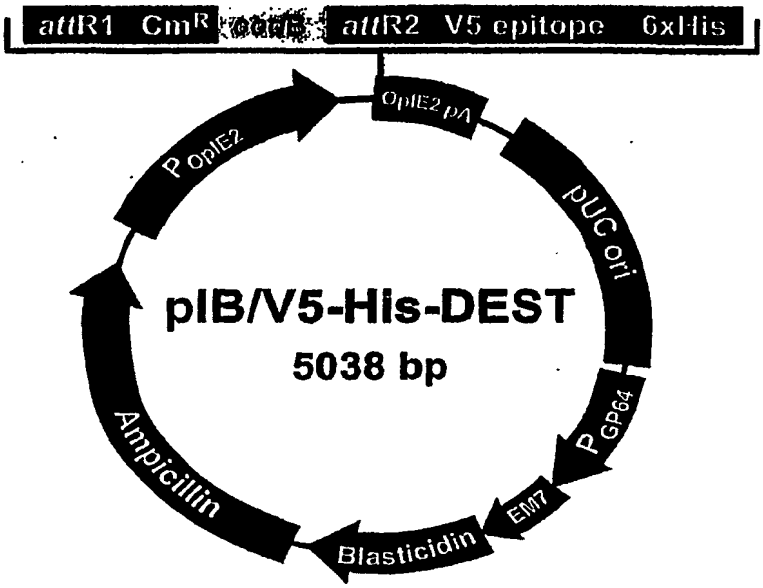

attR1
Cm^R
attR2
V5 epitope
6xHis
OpIE2 pA
pUC ori
P GP64
EM7
Blasticidin
Ampicillin
P OpIE2
pIB/V5-His-DEST
5038 bp

FIG. 15

```
1    GGATCATGAT GATAAACAAT GTATGGTGCT AATGTTGCTT CAACAACAAT TCTGTTGAAC

61   TGTGTTTTCA TGTTTGCCAA CAAGCACCTT TATACTCGGT GGCCTCCCCA CCACCAACTT

121  TTTTGCACTG CAAAAAAACA CGCTTTTGCA CGCGGGCCCA TACATAGTAC AAACTCTACG

181  TTTCGTAGAC TATTTTACAT AAATAGTCTA CACCGTTGTA TACGCTCCAA ATACACTACC

241  ACACATTGAA CCTTTTTGCA GTGCAAAAAA GTACGTGTCG GCAGTCACGT AGGCCGGCCT

301  TATCGGGTCG CGTCCTGTCA CGTACGAATC ACATTATCGG ACCGGACGAG TGTTGTCTTA

361  TCGTGACAGG ACGCCAGCTT CCTGTGTTGC TAACCGCAGC CGGACGCAAC TCCTTATCGG

421  AACAGGACGC GCCTCCATAT CAGCCGCGCG TTATCTCATG CGCGTGACCG GACACGAGGC

                                TATA                    Start of Transcription
481  GCCCGTCCCG CTTATCGCGC CTATAAATAC AGCCCGCAAC GATCTGGTAA ACACAGTTGA

541  ACAGCATCTG TTCGAATTTA
```

FIG. 16

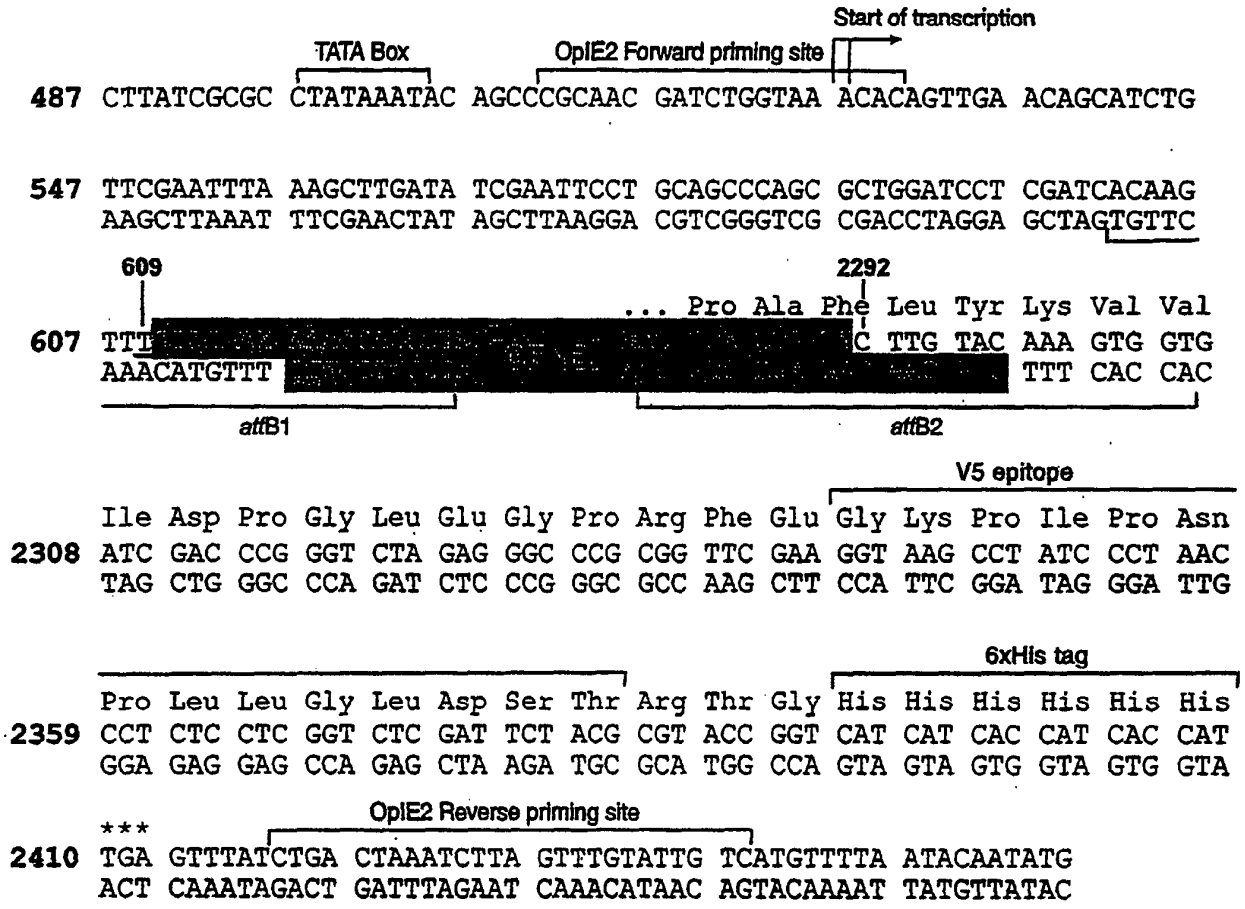

Start of transcription
TATA Box
OpIE2 Forward priming site
487 CTTATCGCGC CTATAAATAC AGCCCGCAAC GATCTGGTAA ACACAGTTGA ACAGCATCTG
547 TTCGAATTTA AAGCTTGATA TCGAATTCCT GCAGCCCAGC GCTGGATCCT CGATCACAAG
AAGCTTAAAT TTCGAACTAT AGCTTAAGGA CGTCGGGTCG CGACCTAGGA GCTAGTGTTC
609
2292
... Pro Ala Phe Leu Tyr Lys Val Val
607 TTT C TTG TAC AAA GTG GTG
AAACATGTTT TTT CAC CAC
attB1
attB2
V5 epitope
Ile Asp Pro Gly Leu Glu Gly Pro Arg Phe Glu Gly Lys Pro Ile Pro Asn
2308 ATC GAC CCG GGT CTA GAG GGC CCG CGG TTC GAA GGT AAG CCT ATC CCT AAC
TAG CTG GGC CCA GAT CTC CCG GGC GCC AAG CTT CCA TTC GGA TAG GGA TTG
6xHis tag
Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly His His His His His His
2359 CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC CAT CAC CAT
GGA GAG GAG CCA GAG CTA AGA TGC GCA TGG CCA GTA GTA GTG GTA GTG GTA
***
OpIE2 Reverse priming site
2410 TGA GTTTATCTGA CTAAATCTTA GTTTGTATTG TCATGTTTTA ATACAATATG
ACT CAAATAGACT GATTTAGAAT CAAACATAAC AGTACAAAAT TATGTTATAC

FIG. 17

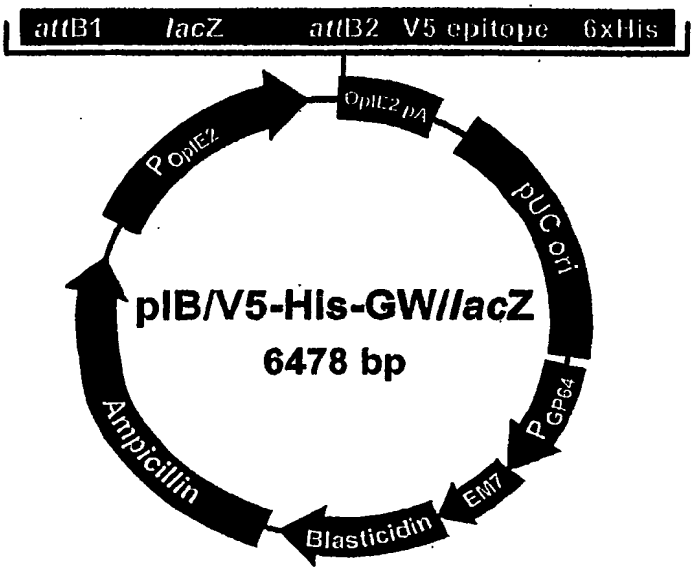

attB1
lacZ
attB2
V5 epitope
6xHis
OpIE2 pA
P OpIE2
pUC ori
pIB/V5-His-GW/lacZ
6478 bp
P GP64
EM7
Blasticidin
Ampicillin

FIG. 18

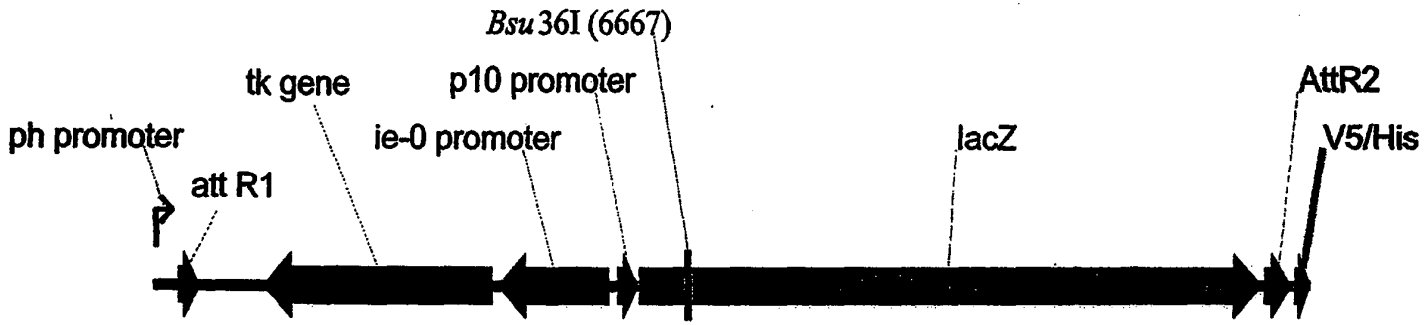

Bsu 36I (6667)
tk gene
p10 promoter
AttR2
ph promoter
ie-0 promoter
lacZ
V5/His
att R1

FIG. 19A

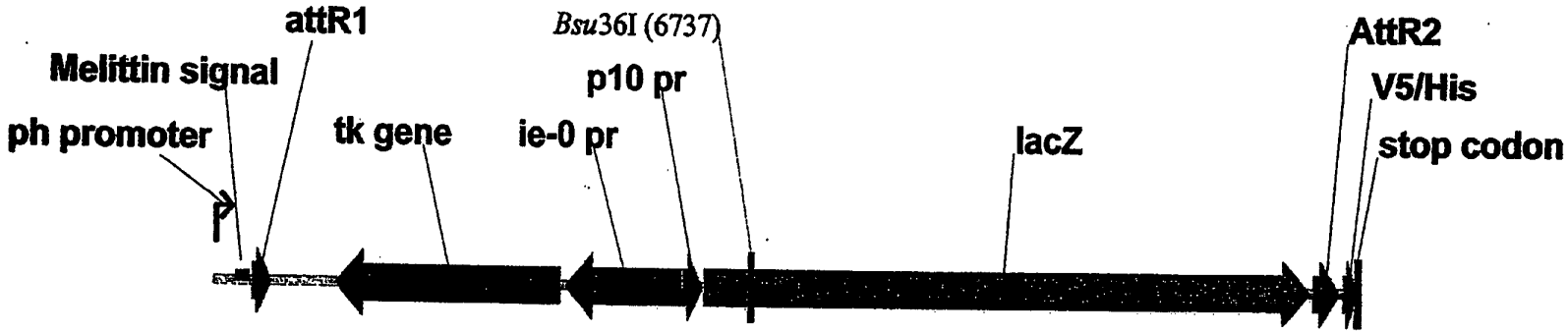

attR1
Melittin signal
ph promoter
tk gene
Bsu36I (6737)
p10 pr
ie-0 pr
lacZ
AttR2
V5/His
stop codon

FIG. 19B

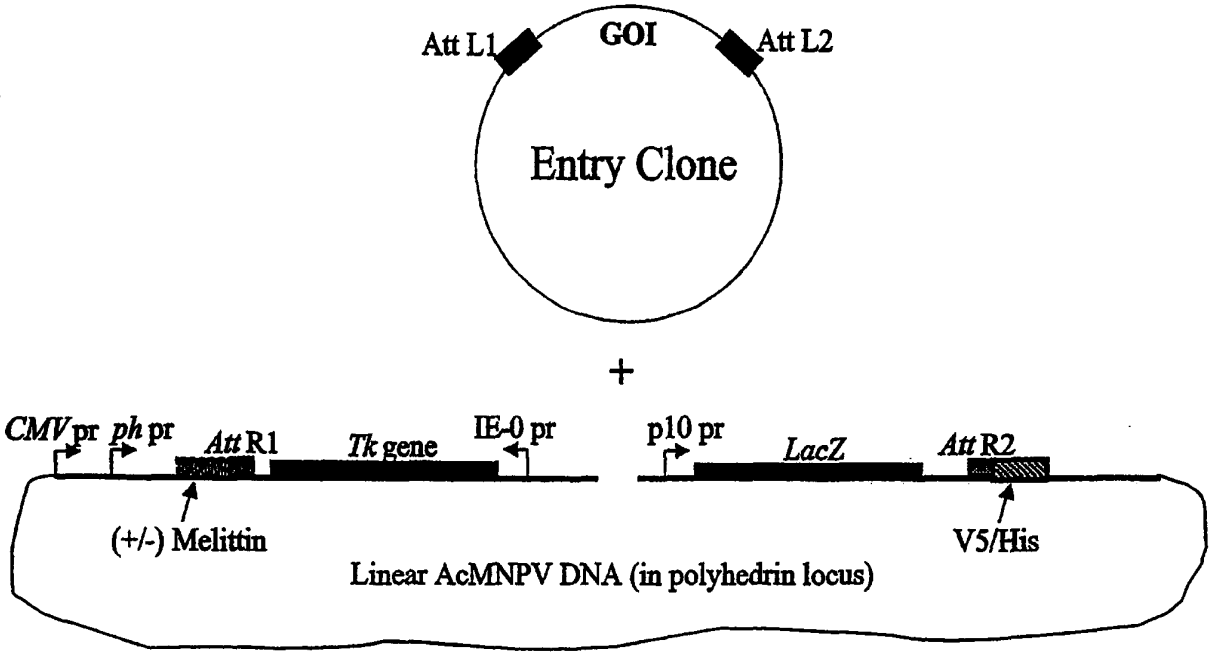
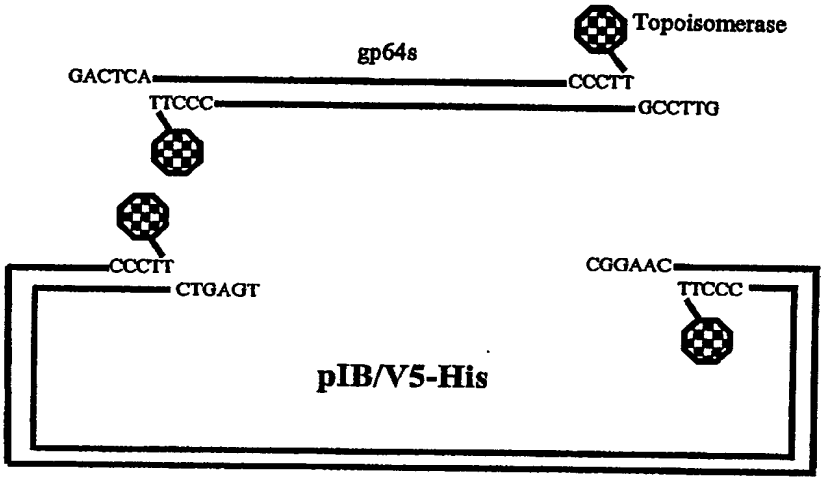

Att L1
GOI
Att L2
Entry Clone
+
CMV pr
ph pr
Att R1
Tk gene
IE-0 pr
p10 pr
LacZ
Att R2
(+/-) Melittin
V5/His
Linear AcMNPV DNA (in polyhedrin locus)

FIG. 20

FIG. 21


Topoisomerase
gp64s
GACTCA
CCCTT
TTCCC
GCCTTG
CCCTT
CTGAGT
CGGAAC
TTCCC
pIB/V5-His

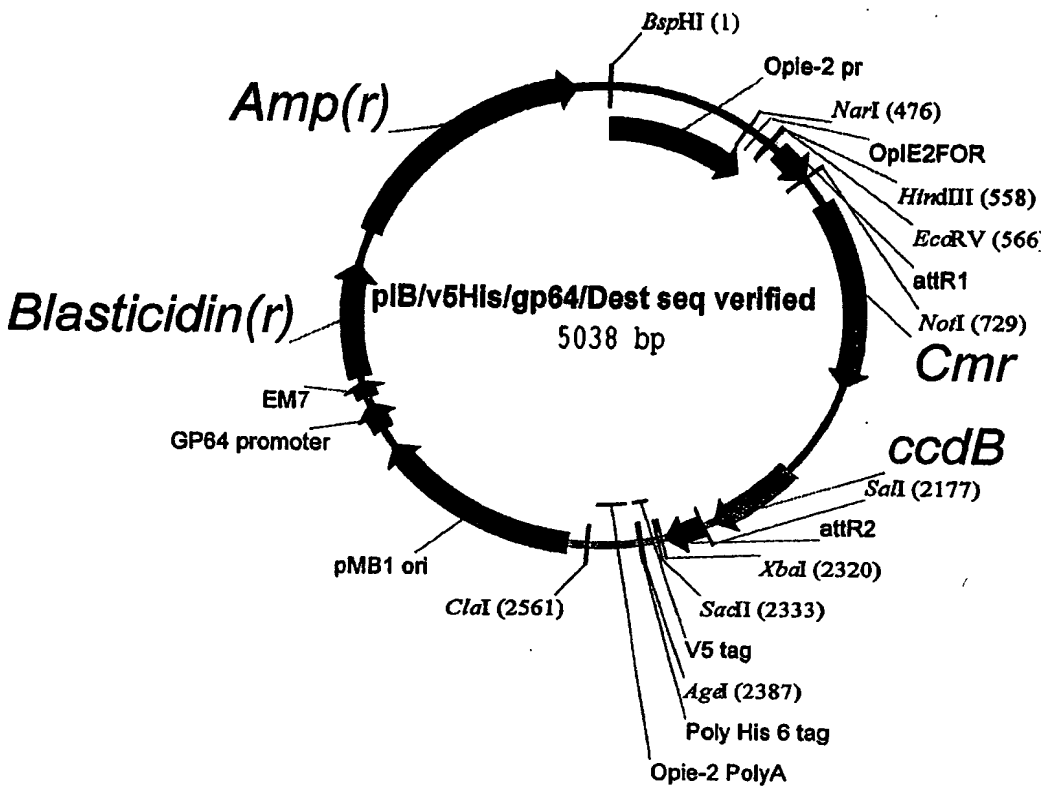

BspHI (1)
Opie-2 pr
NarI (476)
OpIE2FOR
HindIII (558)
EcoRV (566
attR1
NotI (729)
Cmr
ccdB
SalI (2177)
attR2
XbaI (2320)
SacI (2333)
V5 tag
AgeI (2387)
Poly His 6 tag
Opie-2 PolyA
ClaI (2561)
pMB1 ori
GP64 promoter
EM7
Blasticidin(r)
Amp(r)
pIB/v5His/gp64/Dest seq verified
5038 bp

FIG. 22

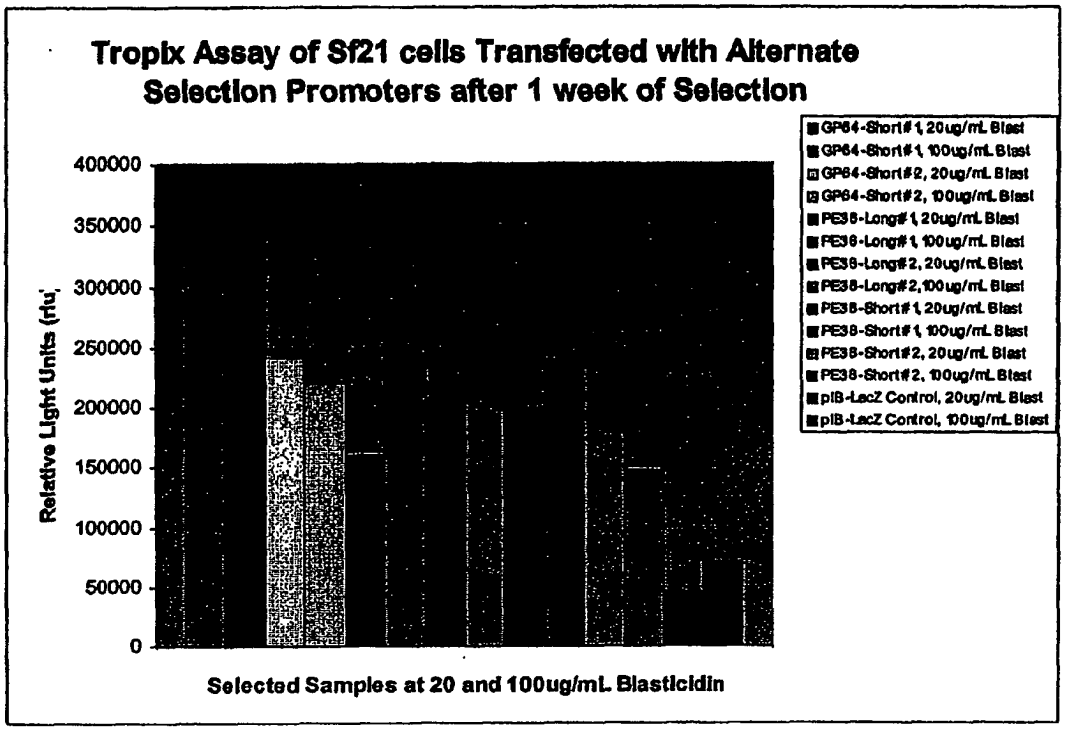

Tropix Assay of Sf21 cells Transfected with Alternate Selection Promoters after 1 week of Selection
Relative Light Units (rlu)
400000
350000
300000
250000
200000
150000
100000
50000
0
Selected Samples at 20 and 100ug/mL Blasticidin
GP64-Short#1, 20ug/mL Blast
GP64-Short#1, 100ug/mL Blast
GP64-Short#2, 20ug/mL Blast
GP64-Short#2, 100ug/mL Blast
PE38-Long#1, 20ug/mL Blast
PE38-Long#1, 100ug/mL Blast
PE38-Long#2, 20ug/mL Blast
PE38-Long#2, 100ug/mL Blast
PE38-Short#1, 20ug/mL Blast
PE38-Short#1, 100ug/mL Blast
PE38-Short#2, 20ug/mL Blast
PE38-Short#2, 100ug/mL Blast
pIB-LacZ Control, 20ug/mL Blast
pIB-LacZ Control, 100ug/mL Blast

FIG. 23

FIG. 24

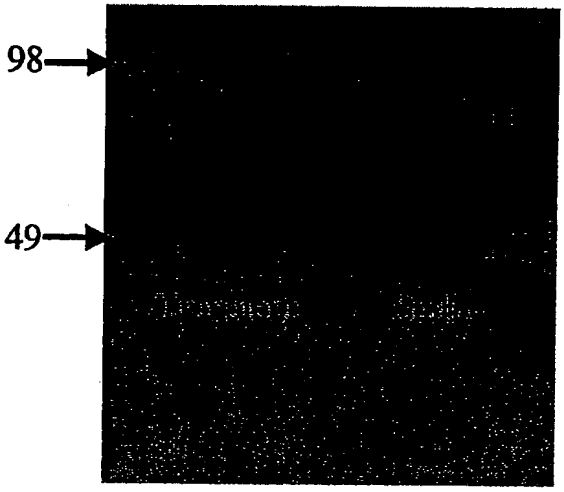
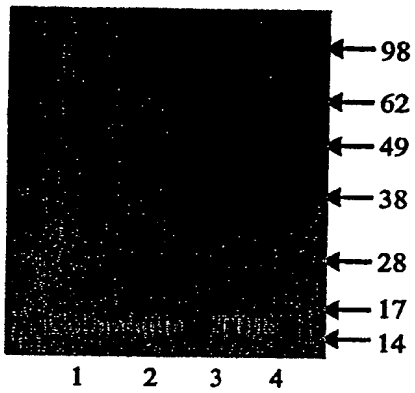


FIG. 25

A.



B.

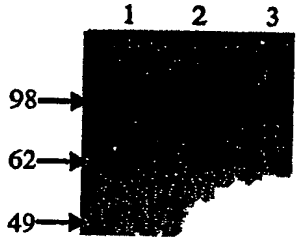


FIG. 26

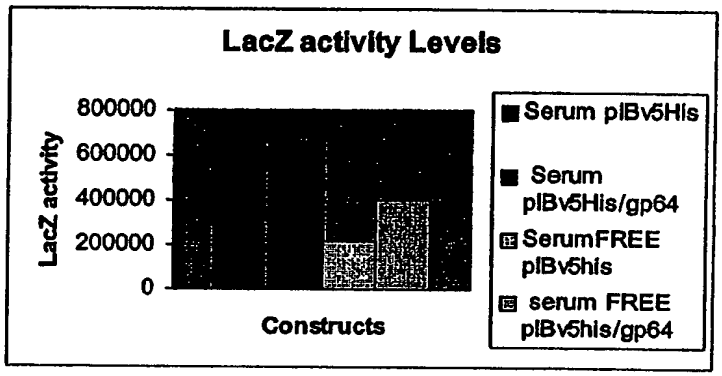

LacZ activity Levels
800000
600000
400000
200000
0
LacZ activity
Constructs
Serum pIBv5His
Serum pIBv5His/gp64
SerumFREE pIBv5his
serum FREE pIBv5his/gp64

FIG. 27A

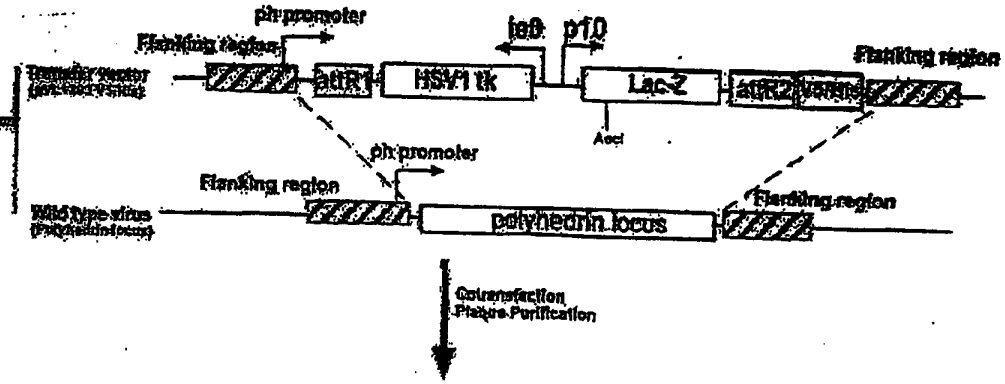
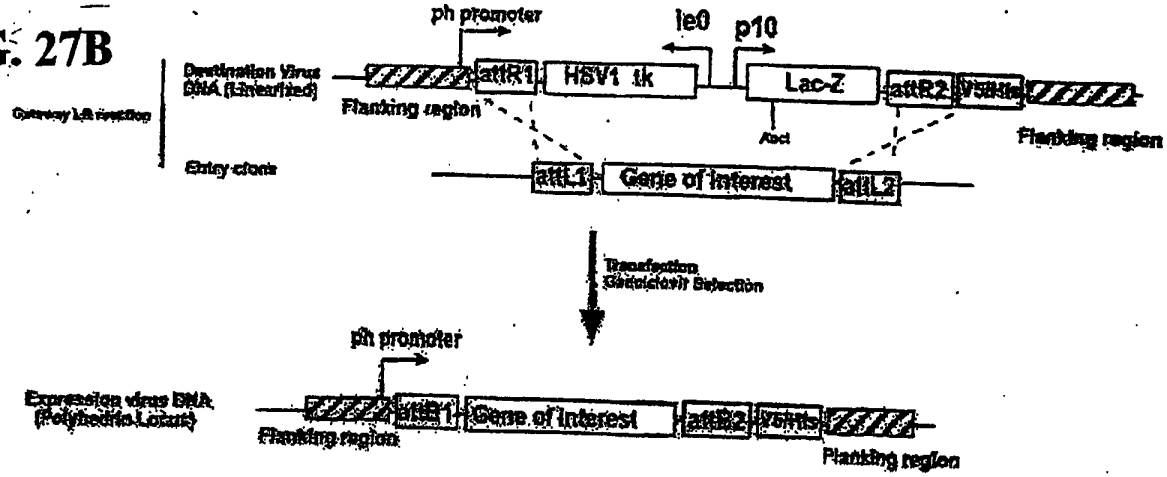


FIG. 27B



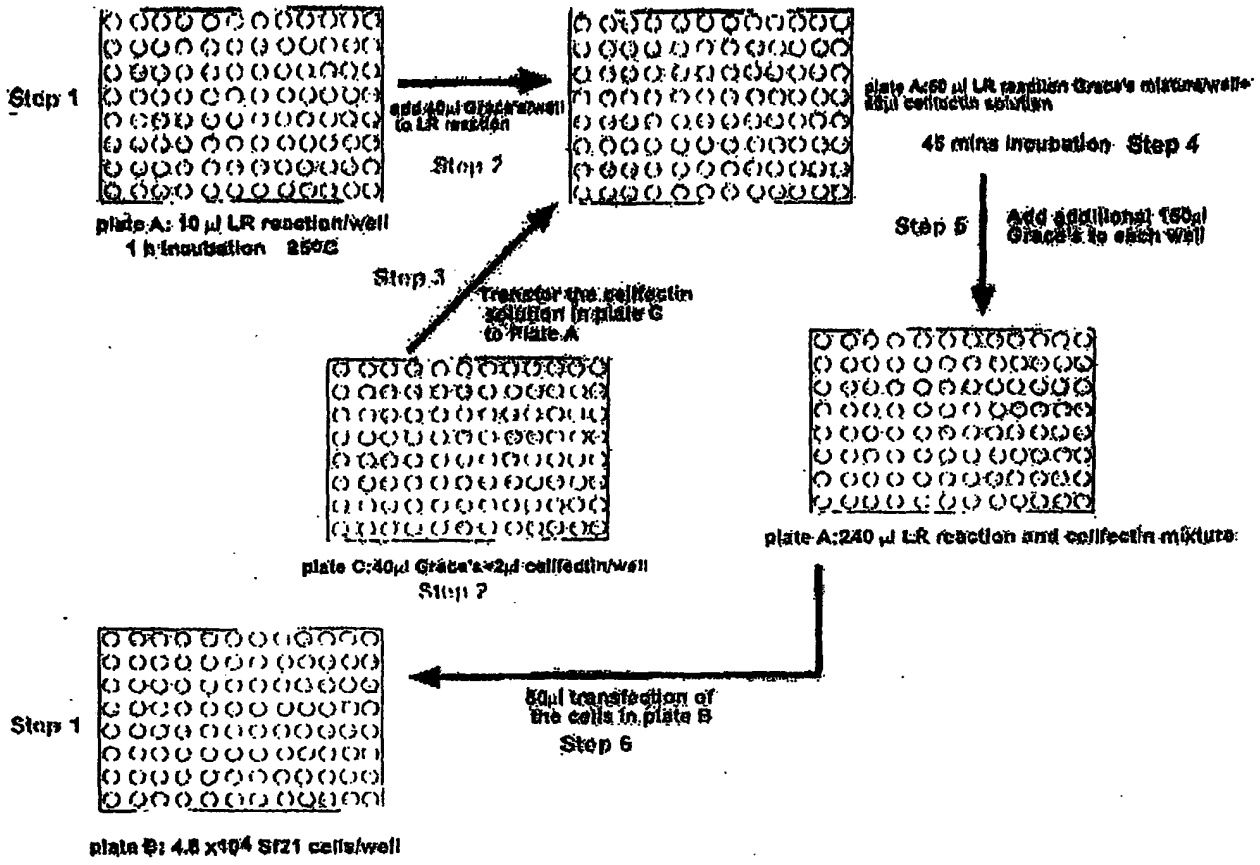

Step 1
plate A: 10 µl LR reaction/well
1 h incubation 25°C
add 40µl Grace's/well to LR reaction
Step 2
plate A:60 µl LR reaction Grace's mixture/well+ 50µl cellfectin solution
45 mins incubation Step 4
Step 5
Add additional 150µl Grace's to each well
Step 3
Transfer the cellfectin solution in plate C to Plate A
plate C:40µl Grace's+2µl cellfectin/well
Step 7
plate A:240 µl LR reaction and cellfectin mixture
50µl transfection of the cells in plate B
Step 6
Step 1
plate B: 4.8 x10^4 Sf21 cells/well

FIG. 28

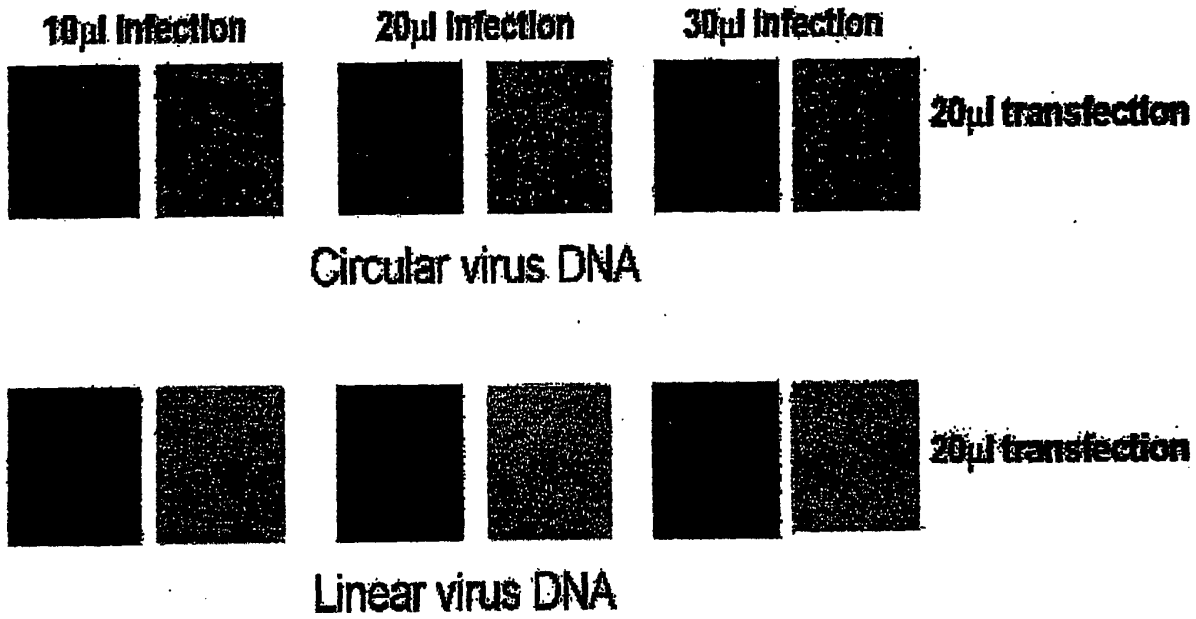

10μl infection
20μl infection
30μl infection
20μl transfection
Circular virus DNA
20μl transfection
Linear virus DNA

FIG. 29

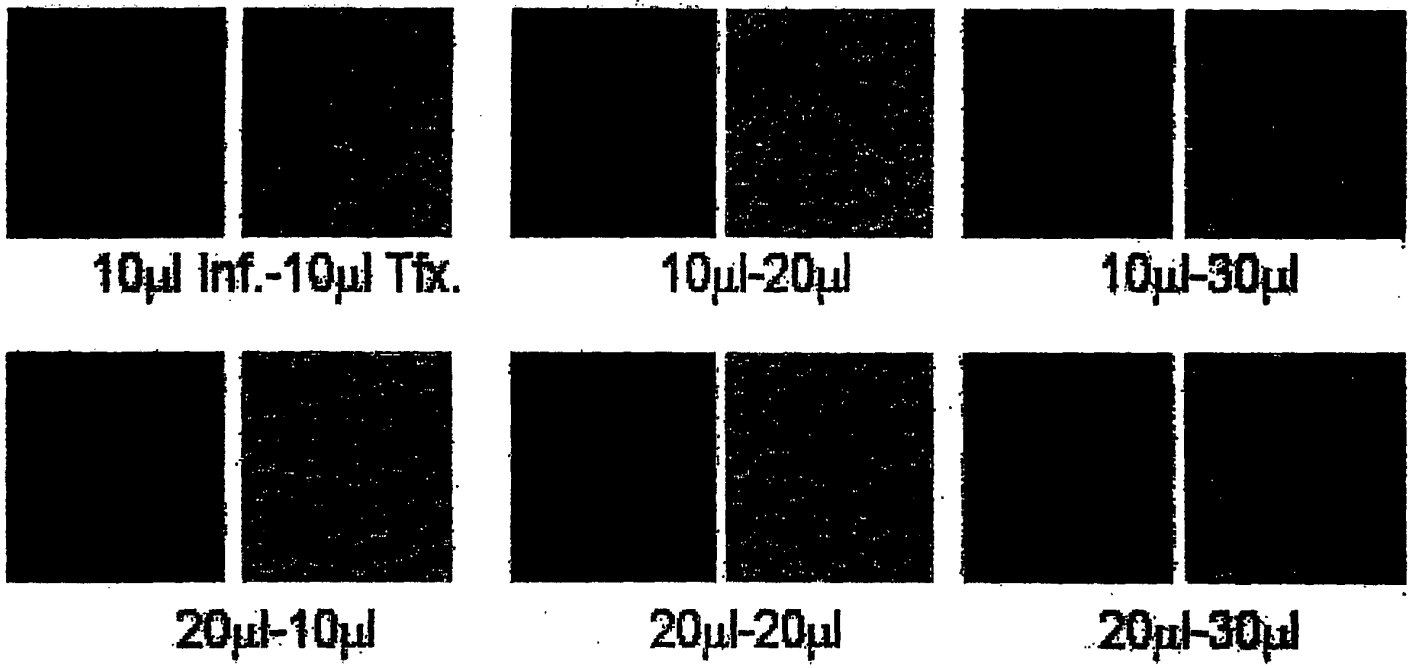

10µl Inf.-10µl Tfx.
10µl-20µl
10µl-30µl
20µl-10µl
20µl-20µl
20µl-30µl

FIG. 30

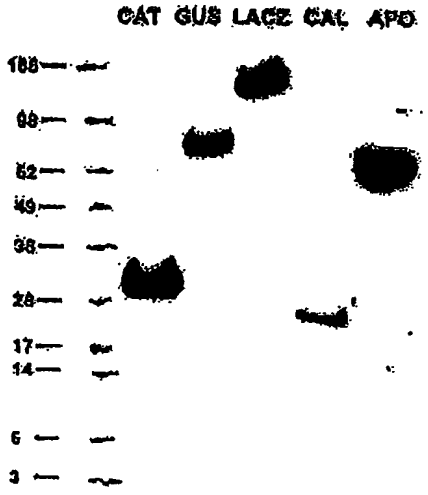


FIG. 31

Titer pfu/ml / Virus	Transfect.	Infect.
Baculodirect™	$9.8X10^6$	$6.9X10^8$
Bac to Bac	$6.9X10^6$	$7.2X10^8$
MaxBac	N/A	$3.6X10^8$

Titer comparison of the three viruses by using $TCID_{50}$

Titer pfu/ml / Virus	Transfect.	Infect.
Baculodirect™	$6X10^6$	$3X10^8$
Bac to Bac	$8X10^6$	$5X10^8$
MaxBac	N/A	$3X10^8$

Titer comparison of the three viruses by using plaque assay

FIG. 32

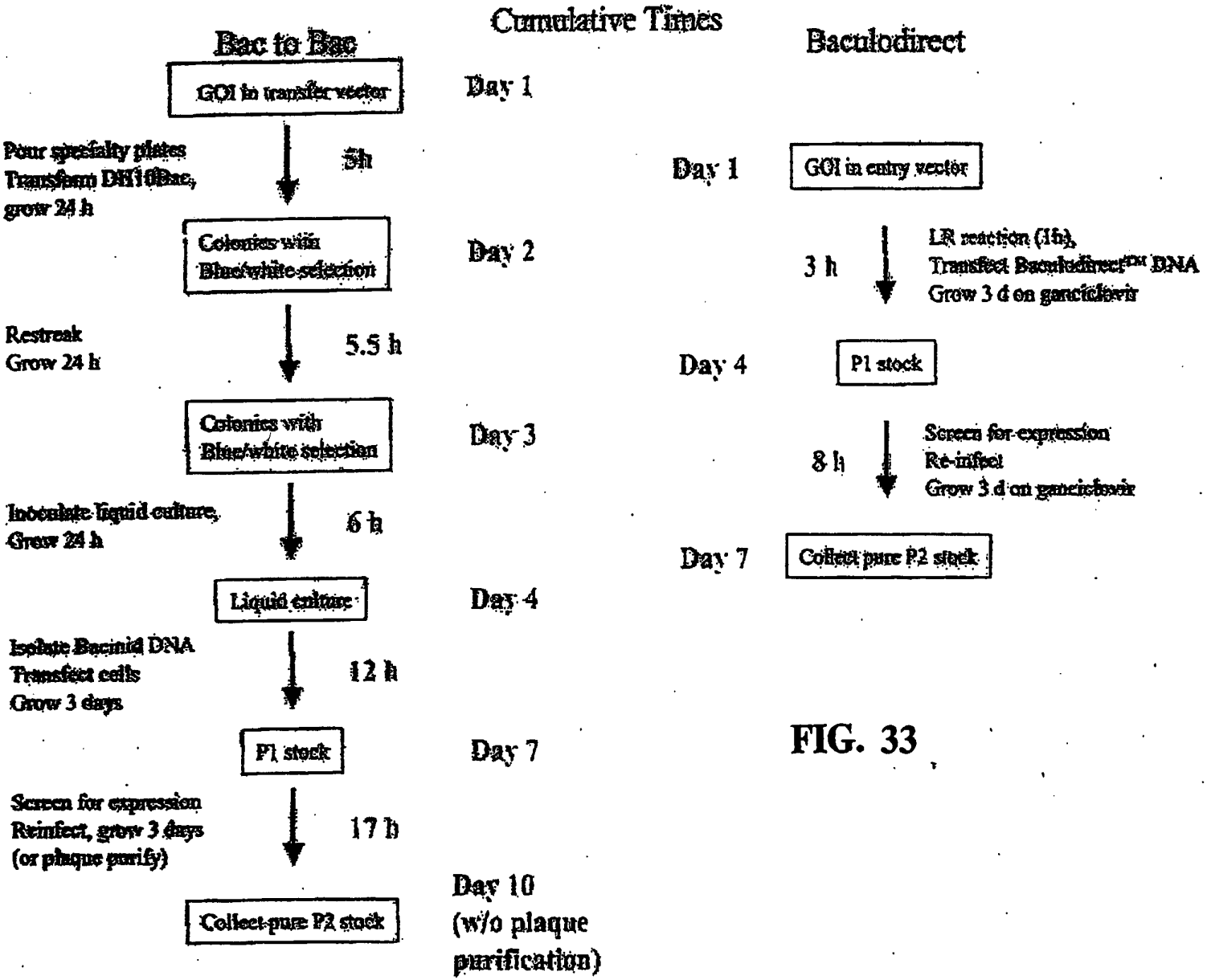

Cumulative Times
Bac to Bac
GOI in transfer vector
Day 1
Pour specialty plates
Transform DH10Bac,
grow 24 h
5h
Colonies with
Blue/white selection
Day 2
Restreak
Grow 24 h
5.5 h
Colonies with
Blue/white selection
Day 3
Inoculate liquid culture,
Grow 24 h
6 h
Liquid culture
Day 4
Isolate Bacmid DNA
Transfect cells
Grow 3 days
12 h
P1 stock
Day 7
Screen for expression
Reinfect, grow 3 days
(or plaque purify)
17 h
Collect pure P2 stock
Day 10
(w/o plaque
purification)
Baculodirect
Day 1
GOI in entry vector
3 h
LR reaction (1h),
Transfect Baculodirect™ DNA
Grow 3 d on ganciclovir
Day 4
P1 stock
8 h
Screen for expression
Re-infect
Grow 3 d on ganciclovir
Day 7
Collect pure P2 stock

FIG. 33

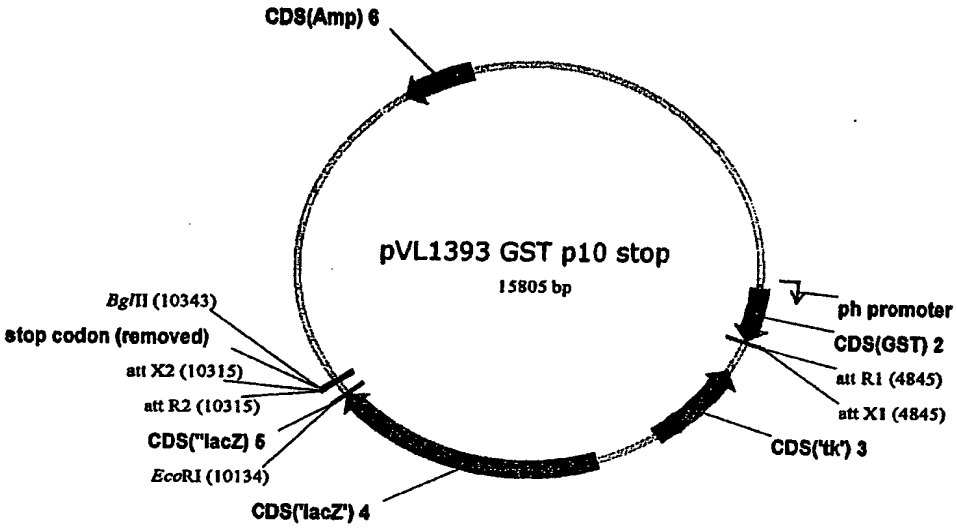
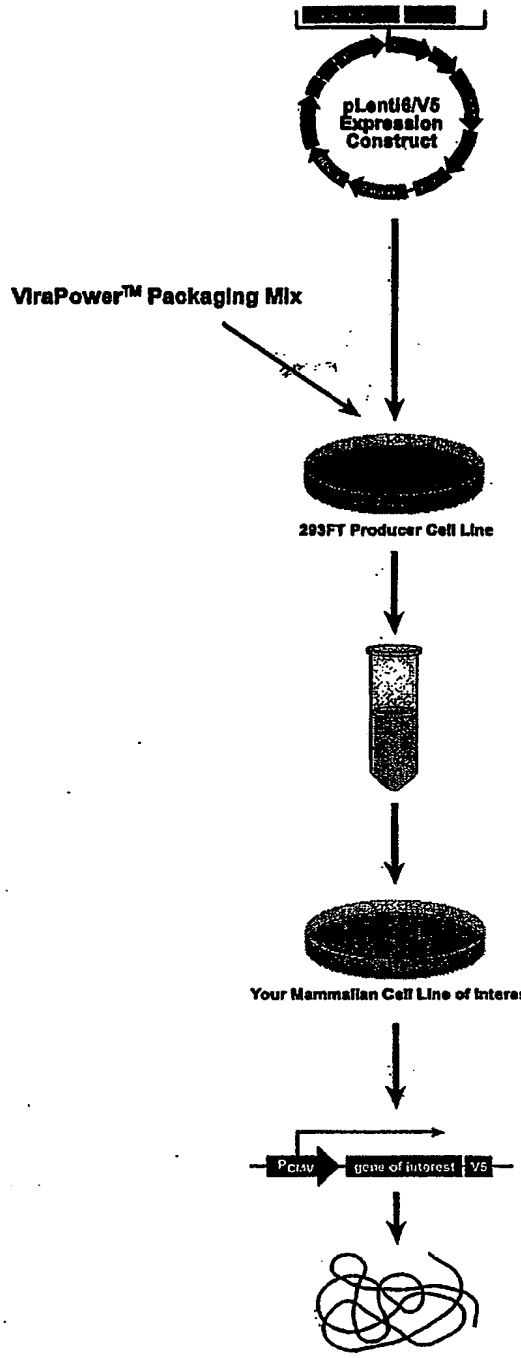

CDS(Amp) 6
pVL1393 GST p10 stop
15805 bp
ph promoter
CDS(GST) 2
att R1 (4845)
att X1 (4845)
CDS('tk') 3
BglII (10343)
stop codon (removed)
att X2 (10315)
att R2 (10315)
CDS("lacZ) 6
EcoRI (10134)
CDS('lacZ') 4

FIG. 34



1. Generate the pLenti6-V5 expression construct containing your gene of interest (see the appropriate pLenti6/V5 expression vector manual).

2. Cotransfect the 293FT producer cell line with your pLenti6/V5 expression construct and the optimized packaging mix.

3. Harvest viral supernatant and determine the titer.

4. Add the viral supernatant to your mammalian cell line of interest. Select for stably transduced cells using blasticidin, if desired.

5. Assay for recombinant protein of interest.

FIG. 35

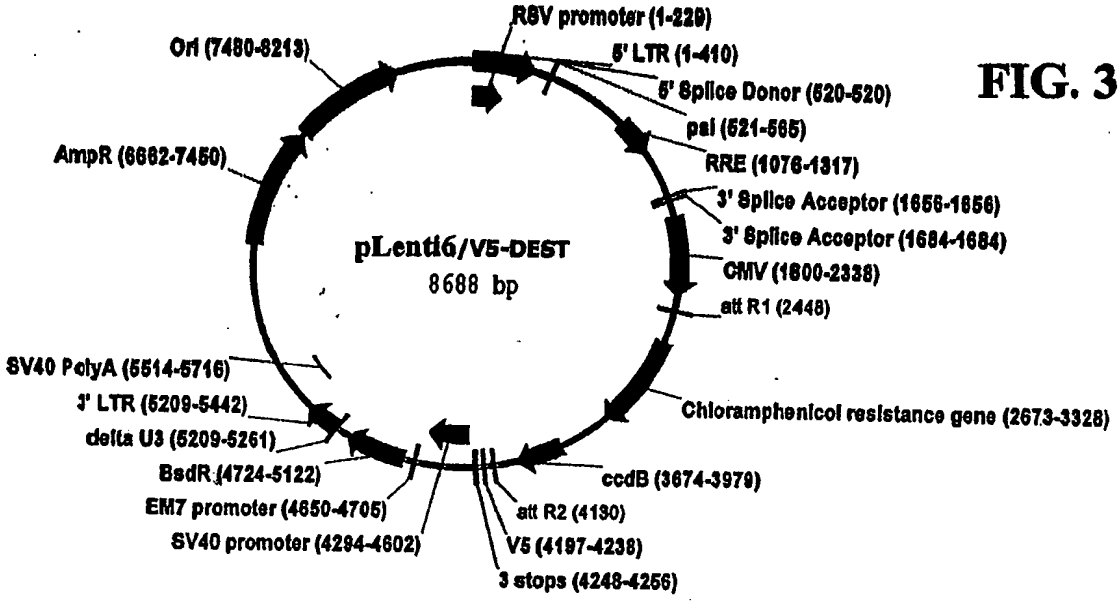


FIG. 36A

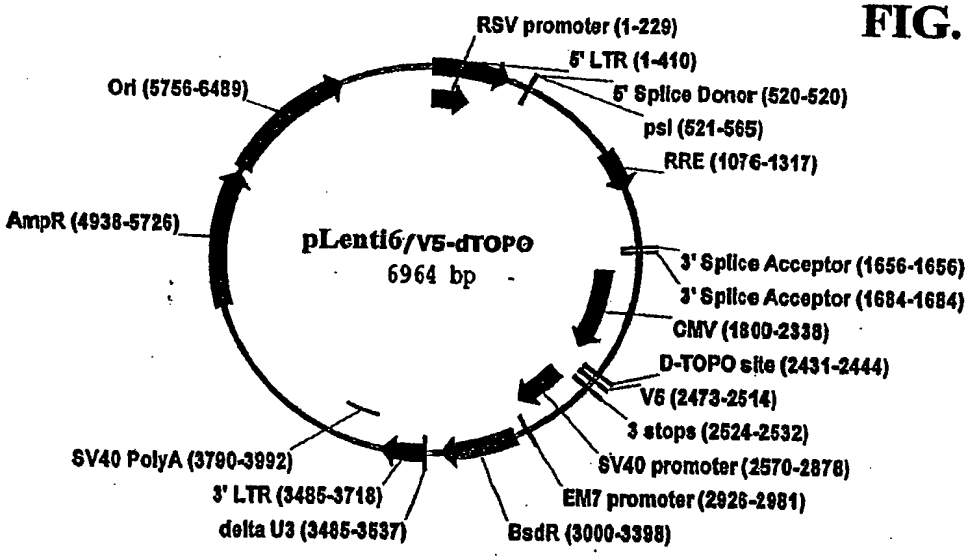


FIG. 36B

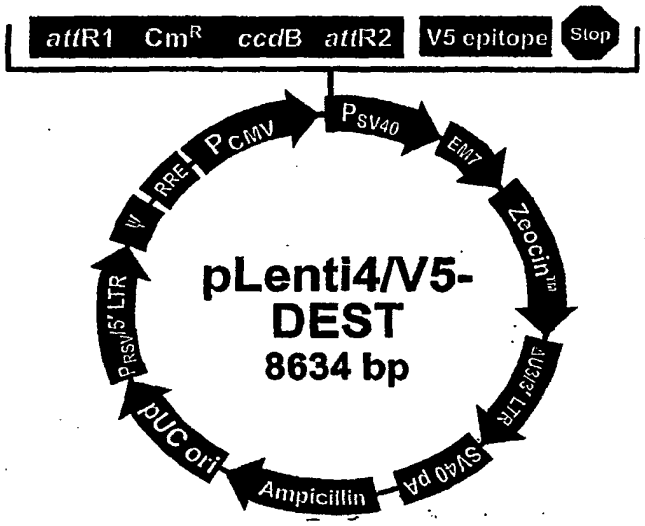

attR1
Cm^R
ccdB
attR2
V5 epitope
Stop
P CMV
P SV40
EM7
Zeocin™
SV40 pA
Ampicillin
pUC ori
P RSV 5' LTR
RRE
Ψ
pLenti4/V5-DEST
8634 bp

FIG. 36C

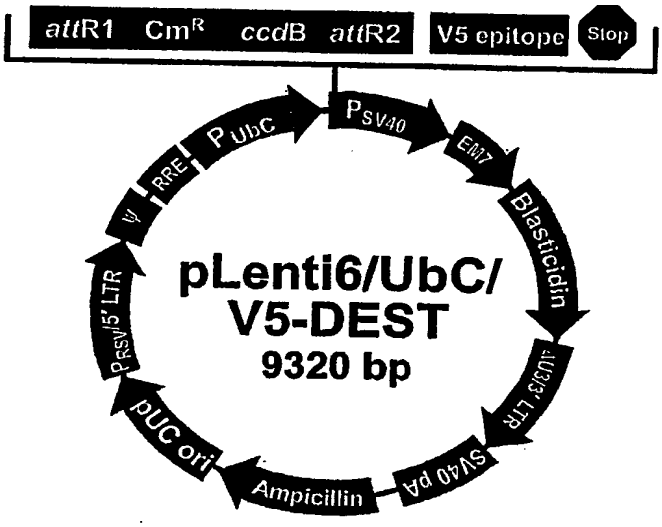

attR1
Cm^R
ccdB
attR2
V5 epitope
Stop
P UbC
RRE
Ψ
P RSV 5' LTR
pUC ori
Ampicillin
SV40 pA
U3 3' LTR
Blasticidin
Em7
P SV40
pLenti6/UbC/
V5-DEST
9320 bp

FIG. 36D

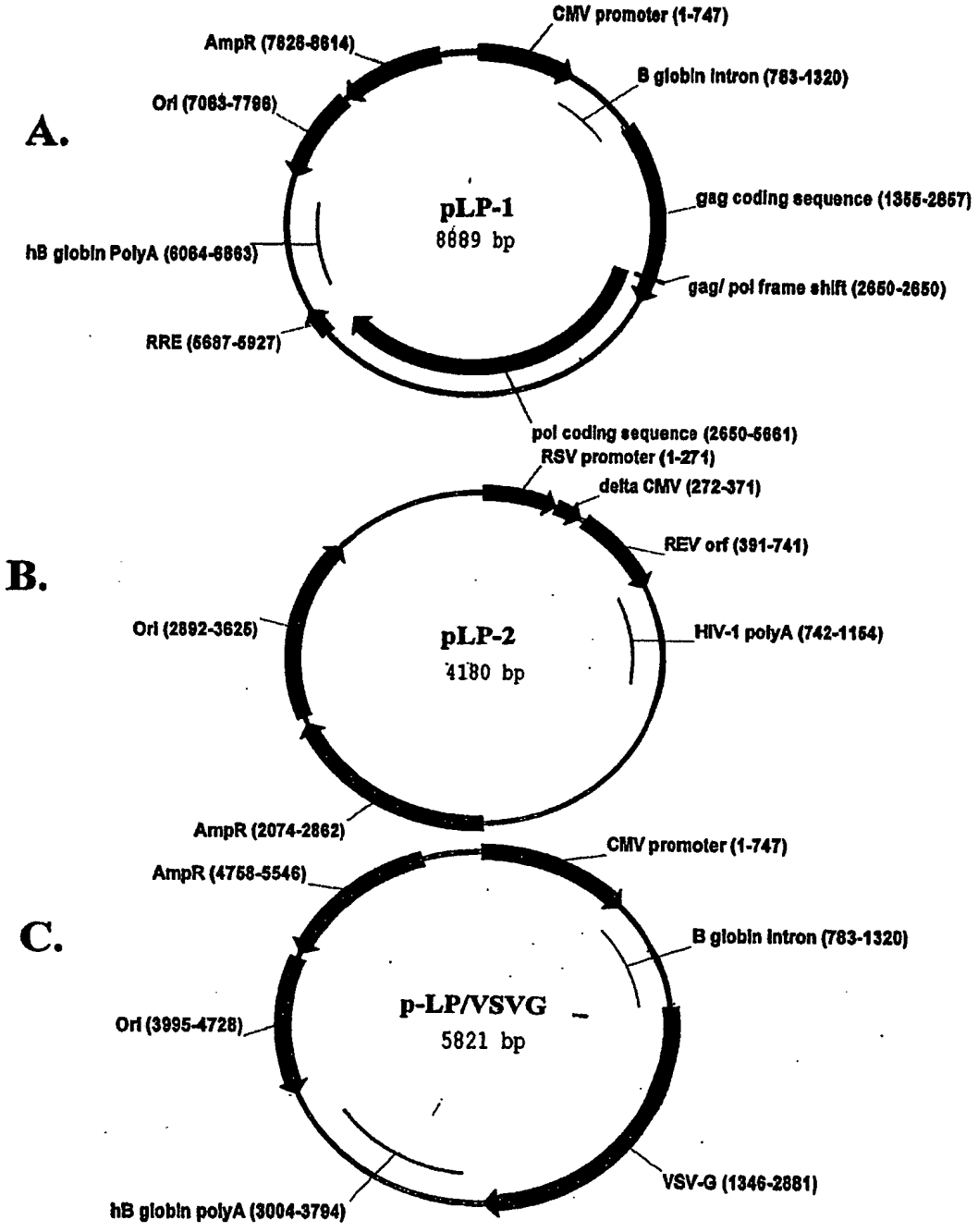
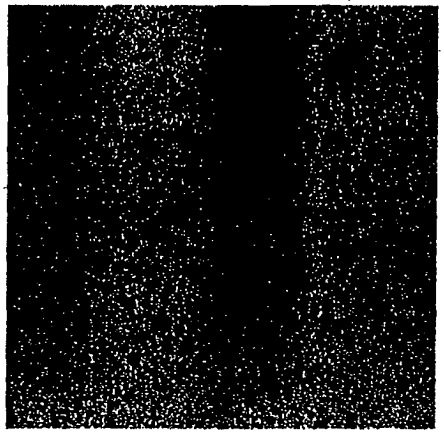

A.
CMV promoter (1-747)
AmpR (7826-8614)
B globin intron (783-1320)
Ori (7063-7786)
pLP-1
8889 bp
gag coding sequence (1355-2857)
hB globin PolyA (6064-6863)
gag/ pol frame shift (2650-2650)
RRE (5687-5927)
pol coding sequence (2650-5661)
B.
RSV promoter (1-271)
delta CMV (272-371)
REV orf (391-741)
Ori (2892-3625)
pLP-2
4180 bp
HIV-1 polyA (742-1164)
AmpR (2074-2862)
C.
CMV promoter (1-747)
AmpR (4758-5546)
B globin intron (783-1320)
p-LP/VSVG
5821 bp
Ori (3995-4728)
VSV-G (1346-2881)
hB globin polyA (3004-3784)

FIG. 37

A.



B.

LR reaction / [Bsd] in plate	DEST alone	DEST + CAT
No Bsd	24	320[1] (12/24 = 50%)[2]
50 ug/ml Bsd	0	162 (24/24 = 100%)[2]

[1]see photo above
[2]percentage of correct colonies

FIG. 38

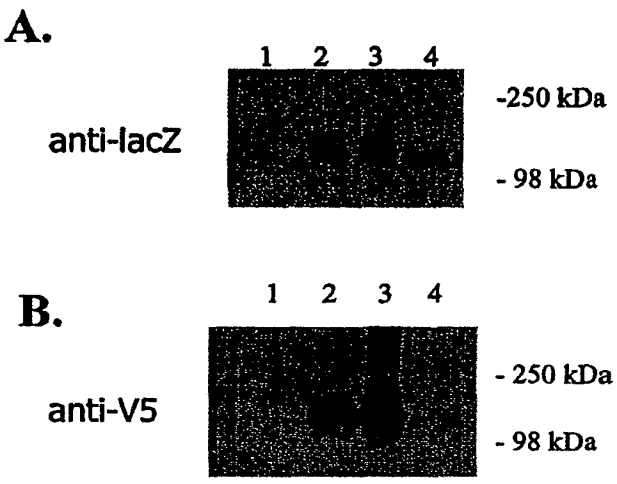


FIG. 39

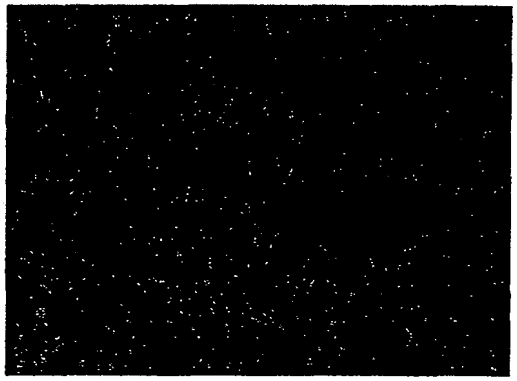
Examples of Production Titers (Bsd^R cfu/ml)

	Empty	LacZ	GFP	CAT	PKC
Exp 1	6×10^6	5×10^5	4×10^6	n.d.	n.d.
Exp 2	3×10^7	3×10^5	6×10^6	8×10^6	n.d.
Exp 3	7×10^6	6×10^5	2×10^6	1×10^7	3×10^6
AVG	$\mathbf{1.4 \times 10^7}$	$\mathbf{4.7 \times 10^5}$	$\mathbf{4 \times 10^6}$	$\mathbf{9 \times 10^6}$	$\mathbf{3 \times 10^6}$

n.d. = not determined

FIG. 40

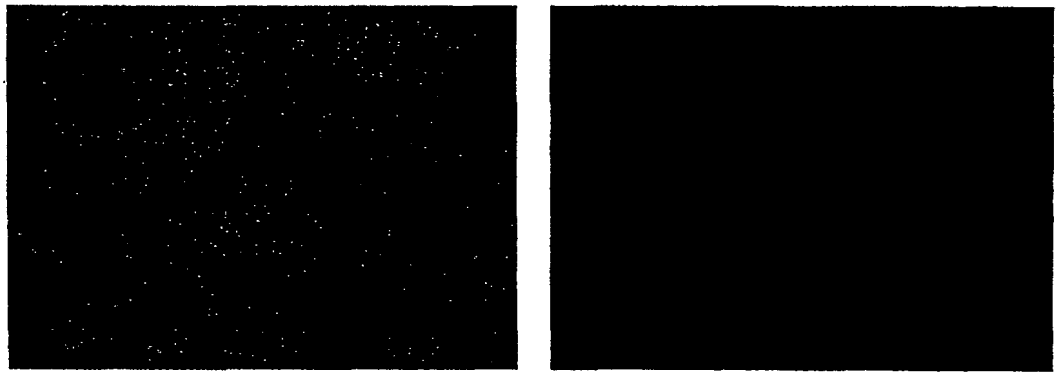
A.



pLenti6/V5-GW/lacZ

B.

C.



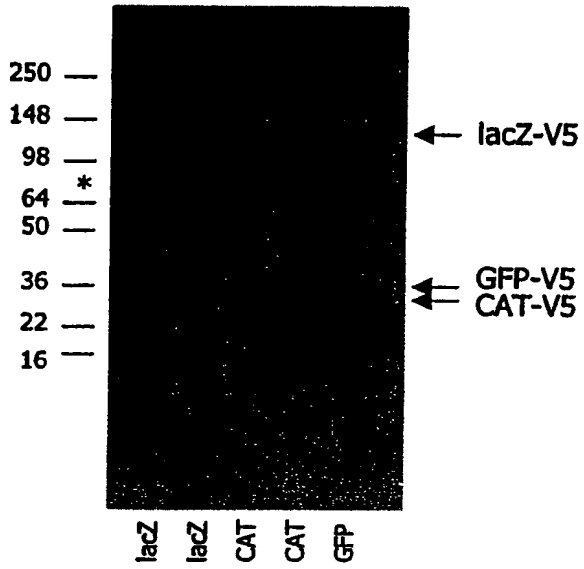
Brightfield

Fluorescent

pLenti6/V5-dT/GFP

FIG. 41

A.


250
148
98
64
50
36
22
16
*
lacZ-V5
GFP-V5
CAT-V5
lacZ
lacZ
CAT
CAT
GFP

B.

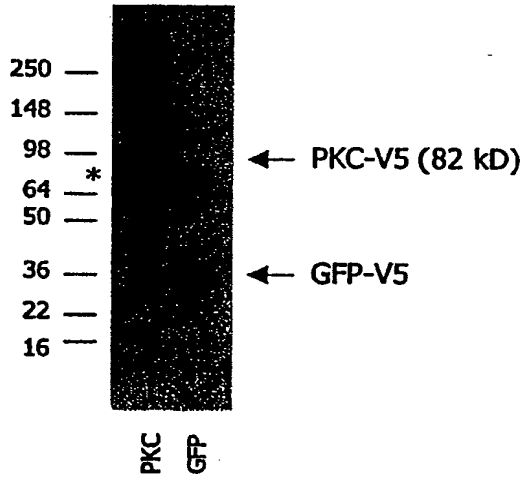
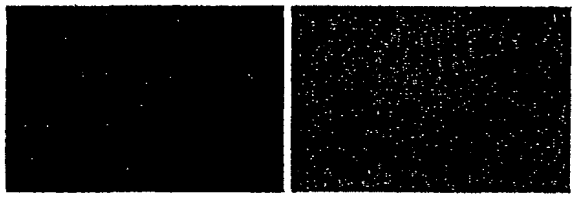

250
148
98
64
50
36
22
16
*
PKC-V5 (82 kD)
GFP-V5
PKC
GFP

FIG. 42

A.

MOI 0 MOI 0.05 MOI 0.1



MOI 0.5 MOI 1

B.

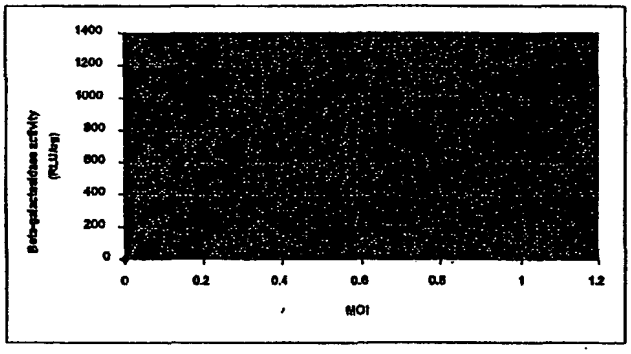
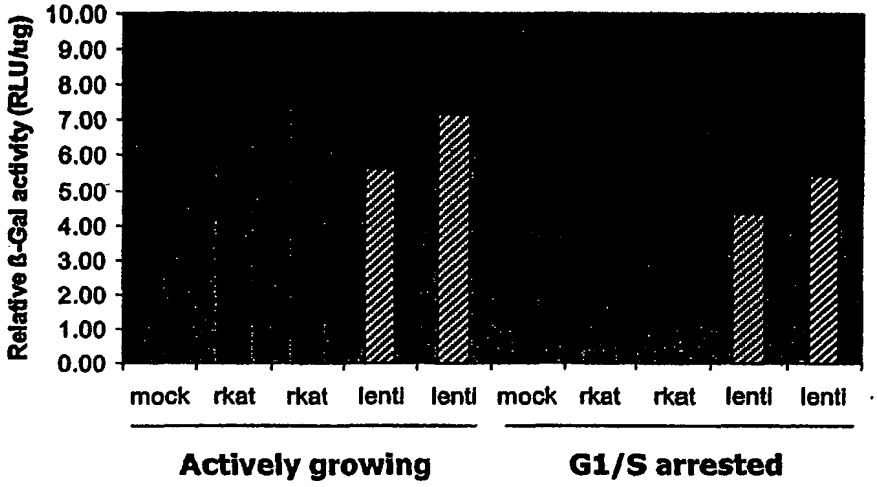


FIG. 43

A.


Relative ß-Gal activity (RLU/ug)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
mock
rkat
rkat
lenti
lenti
mock
rkat
rkat
lenti
lenti
Actively growing
G1/S arrested

B.

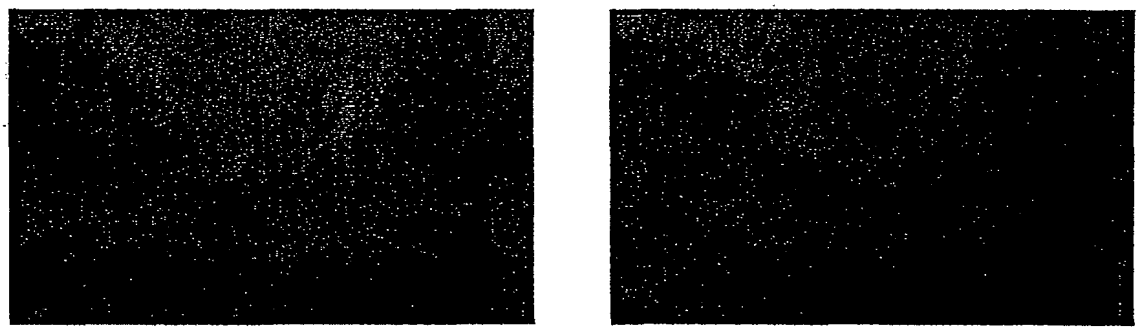

rKAT6-lacZ retrovirus
pLenti6/V5-GW/lacZ

FIG. 44

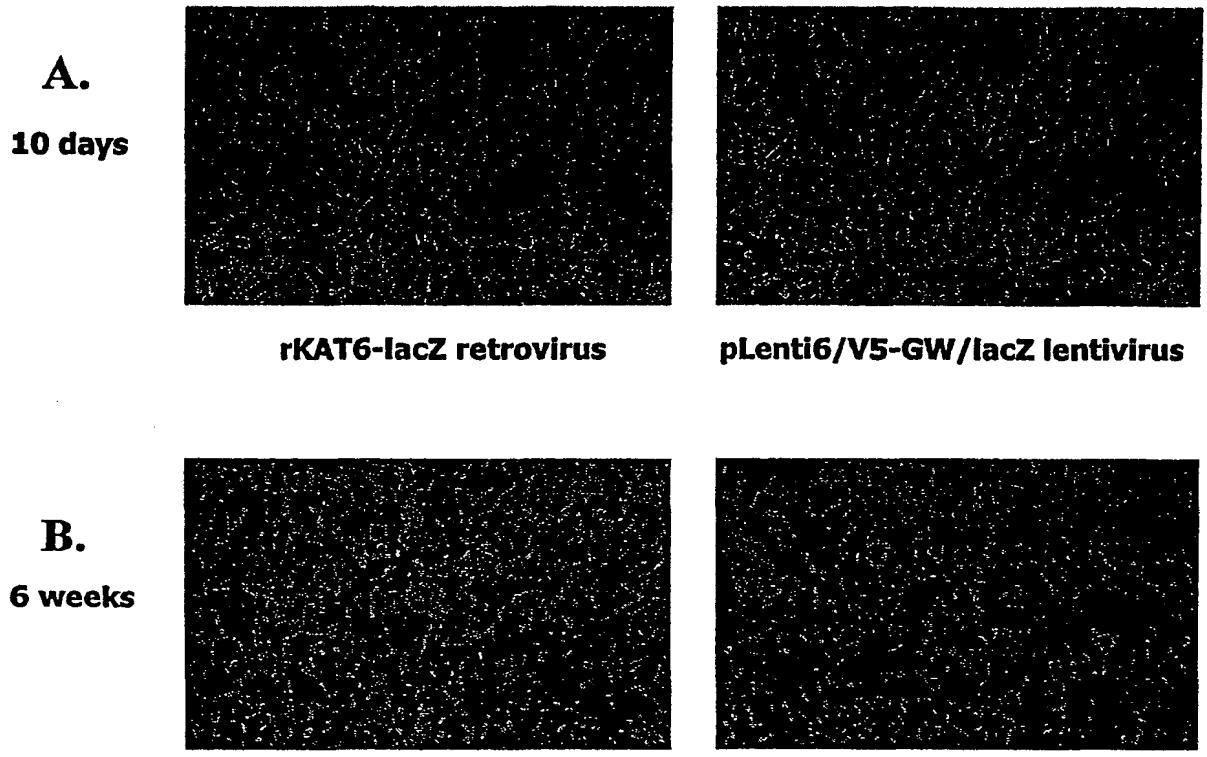

A.
10 days
rKAT6-lacZ retrovirus
pLenti6/V5-GW/lacZ lentivirus
B.
6 weeks
rKAT6-lacZ retrovirus
pLenti6/V5-GW/lacZ lentivirus

FIG. 45

FIG. 46A

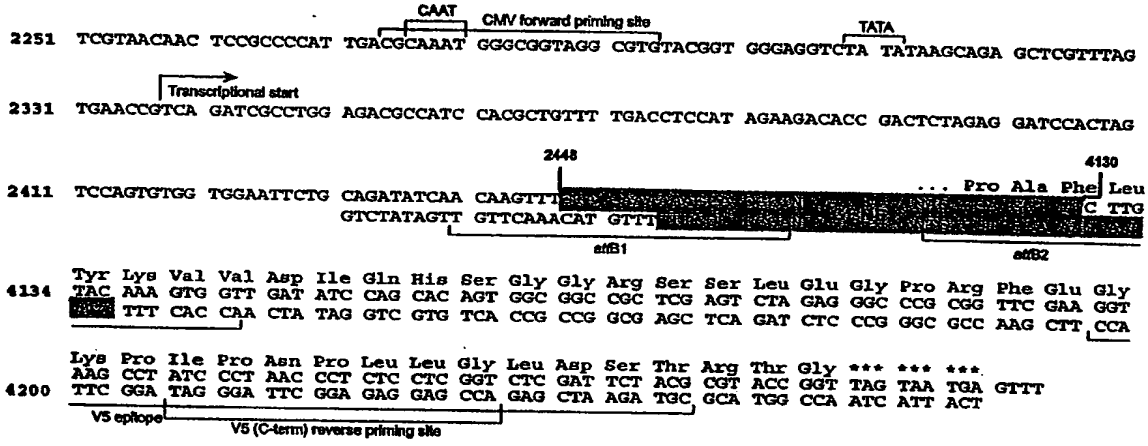

CAAT
CMV forward priming site
TATA
Transcriptional start
2448
4130
attB1
attB2
2251 TCGTAACAAC TCCGCCCCAT TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTCGTTTAG
2331 TGAACCGTCA GATCGCCTGG AGACGCCATC CACGCTGTTT TGACCTCCAT AGAAGACACC GACTCTAGAG GATCCACTAG
2411 TCCAGTGTGG TGGAATTCTG CAGATATCAA CAAGTTT ... Pro Ala Phe Leu
GTCTATAGTT GTTCAAACAT GTTT C TTG
4134 Tyr Lys Val Val Asp Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly
TAC AAA GTG GTT GAT ATC CAG CAC AGT GGC GGC CGC TCG AGT CTA GAG GGC CCG CGG TTC GAA GGT
TTT CAC CAA CTA TAG GTC GTG TCA CCG CCG GCG AGC TCA GAT CTC CCG GGC GCC AAG CTT CCA
4200 Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly *** *** ***
AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTT
TTC GGA TAG GGA TTC GGA GAG GAG CCA GAG CTA AGA TGC GCA TGG CCA ATC ATT ACT
V5 epitope
V5 (C-term) reverse priming site

FIG. 46B

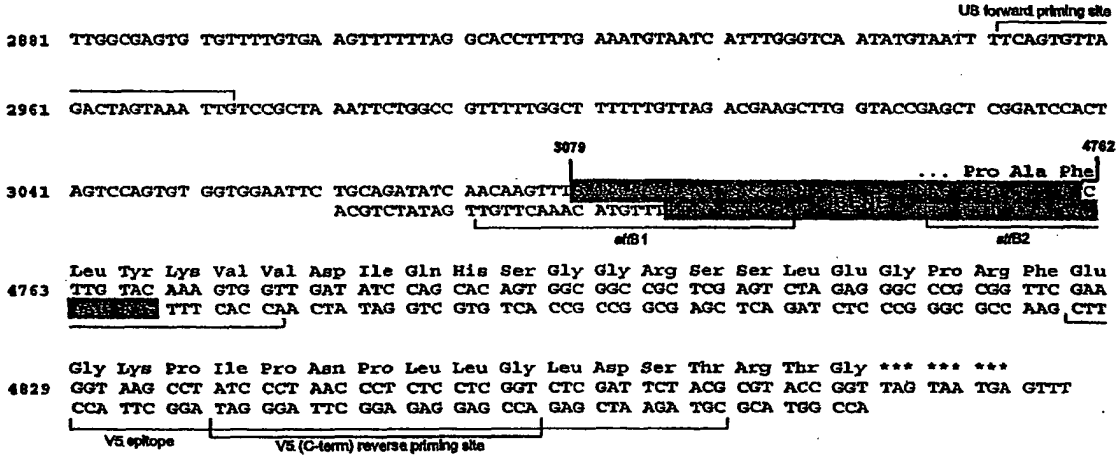
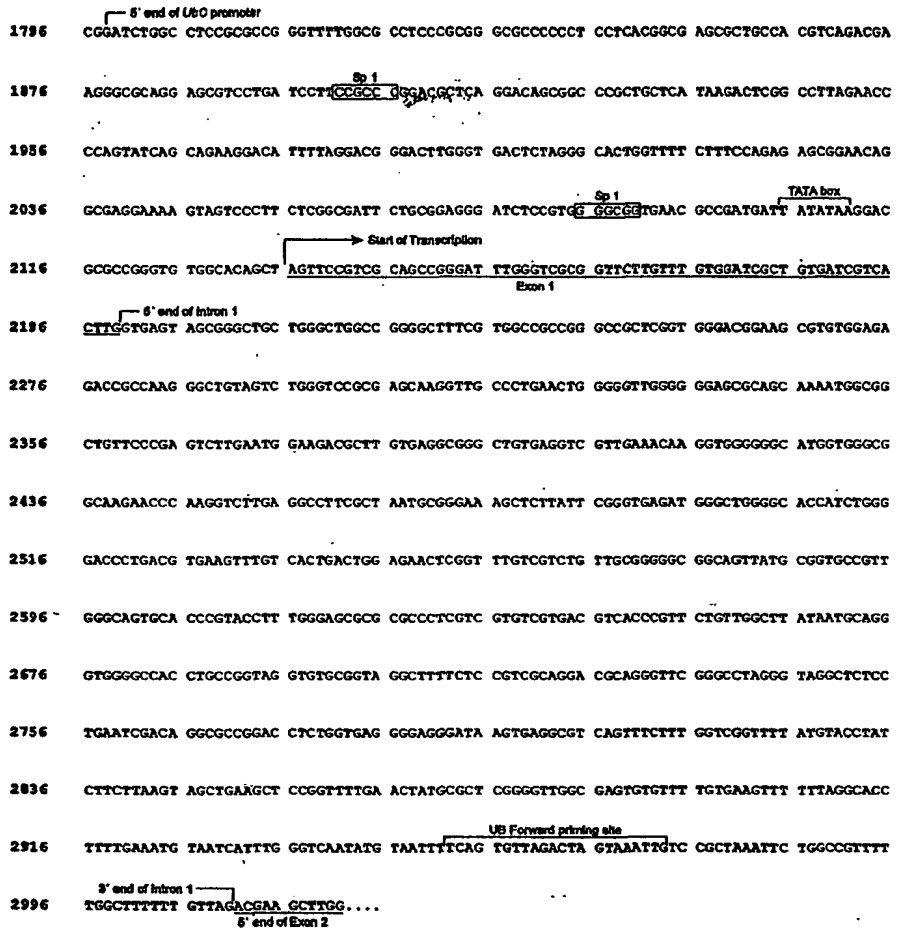

UB forward priming site
2881 TTGGCGAGTG TGTTTTGTGA AGTTTTTTAG GCACCTTTTG AAATGTAATC ATTTGGGTCA ATATGTAATT TTCAGTGTTA
2961 GACTAGTAAA TTGTCCGCTA AATTCTGGCC GTTTTTGGCT TTTTTGTTAG ACGAAGCTTG GTACCGAGCT CGGATCCACT
3079
4762
... Pro Ala Phe
3041 AGTCCAGTGT GGTGGAATTC TGCAGATATC AACAAGTTT
ACGTCTATAG TTGTTCAAAC ATGTTT
C
attB1
attB2
Leu Tyr Lys Val Val Asp Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu
4763 TTG TAC AAA GTG GTT GAT ATC CAG CAC AGT GGC GGC CGC TCG AGT CTA GAG GGC CCG CGG TTC GAA
TTT CAC CAA CTA TAG GTC GTG TCA CCG CCG GCG AGC TCA GAT CTC CCG GGC GCC AAG CTT
Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly *** *** ***
4829 GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTT
CCA TTC GGA TAG GGA TTC GGA GAG GAG CCA GAG CTA AGA TGC GCA TGG CCA
V5 epitope
V5 (C-term) reverse priming site

FIG. 46C



1796 CGGATCTGGC CTCCGCGCCG GGTTTTGGCG CCTCCCGCGG GCGCCCCCCT CCTCACGGCG AGCGCTGCCA CGTCAGACGA

1876 AGGGCGCAGG AGCGTCCTGA TCCTTCCGCC GGGACGCTCA GGACAGCGGC CCGCTGCTCA TAAGACTCGG CCTTAGAACC

1956 CCAGTATCAG CAGAAGGACA TTTTAGGACG GGACTTGGGT GACTCTAGGG CACTGGTTTT CTTTCCAGAG AGCGGAACAG

2036 GCGAGGAAAA GTAGTCCCTT CTCGGCGATT CTGCGGAGGG ATCTCCGTGG GGCGGTGAAC GCCGATGATT ATATAAGGAC

2116 GCGCCGGGTG TGGCACAGCT AGTTCCGTCG CAGCCGGGAT TTGGGTCGCG GTTCTTGTTT GTGGATCGCT GTGATCGTCA

2196 CTTGGTGAGT AGCGGGCTGC TGGGCTGGCC GGGGCTTTCG TGGCCGCCGG GCCGCTCGGT GGGACGGAAG CGTGTGGAGA

2276 GACCGCCAAG GGCTGTAGTC TGGGTCCGCG AGCAAGGTTG CCCTGAACTG GGGGTTGGGG GGAGCGCAGC AAAATGGCGG

2356 CTGTTCCCGA GTCTTGAATG GAAGACGCTT GTGAGGCGGG CTGTGAGGTC GTTGAAACAA GGTGGGGGGC ATGGTGGGCG

2436 GCAAGAACCC AAGGTCTTGA GGCCTTCGCT AATGCGGGAA AGCTCTTATT CGGGTGAGAT GGGCTGGGGC ACCATCTGGG

2516 GACCCTGACG TGAAGTTTGT CACTGACTGG AGAACTCGGT TTGTCGTCTG TTGCGGGGGC GGCAGTTATG CGGTGCCGTT

2596 GGGCAGTGCA CCCGTACCTT TGGGAGCGCG CGCCCTCGTC GTGTCGTGAC GTCACCCGTT CTGTTGGCTT ATAATGCAGG

2676 GTGGGGCCAC CTGCCGGTAG GTGTGCGGTA GGCTTTTCTC CGTCGCAGGA CGCAGGGTTC GGGCCTAGGG TAGGCTCTCC

2756 TGAATCGACA GGCGCCGGAC CTCTGGTGAG GGGAGGGATA AGTGAGGCGT CAGTTTCTTT GGTCGGTTTT ATGTACCTAT

2836 CTTCTTAAGT AGCTGAAGCT CCGGTTTTGA ACTATGCGCT CGGGGTTGGC GAGTGTGTTT TGTGAAGTTT TTTAGGCACC

2916 TTTTGAAATG TAATCATTTG GGTCAATATG TAATTTTCAG TGTTAGACTA GTAAATTGTC CGCTAAATTC TGGCCGTTTT

2996 TGGCTTTTTT GTTAGACGAA GCTTGG....

FIG. 47

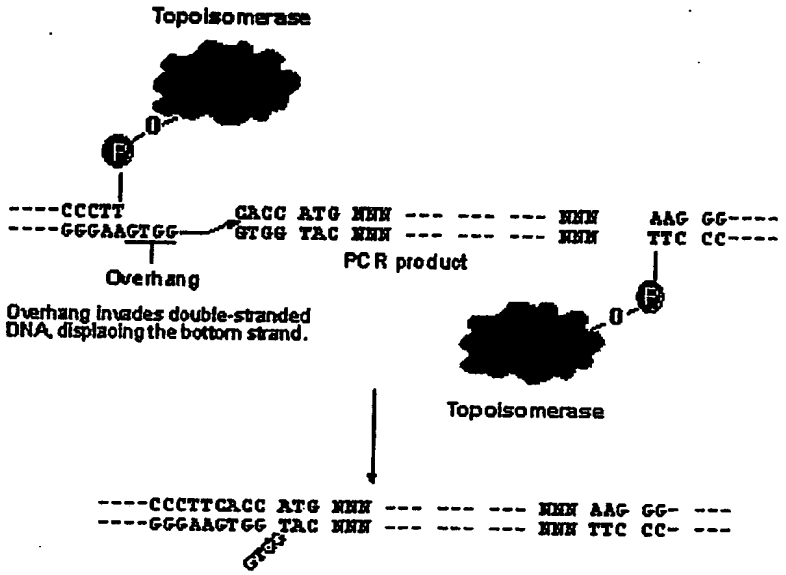

Topoisomerase
----CCCTT
----GGGAAGTGG
CACC ATG NNN --- --- --- NNN
GTGG TAC NNN --- --- --- NNN
AAG GG----
TTC CC----
PCR product
Overhang
Overhang invades double-stranded DNA, displacing the bottom strand.
Topoisomerase
----CCCTTCACC ATG NNN --- --- --- NNN AAG GG- ---
----GGGAAGTGG TAC NNN --- --- --- NNN TTC CC- ---
GTGG

FIG. 48

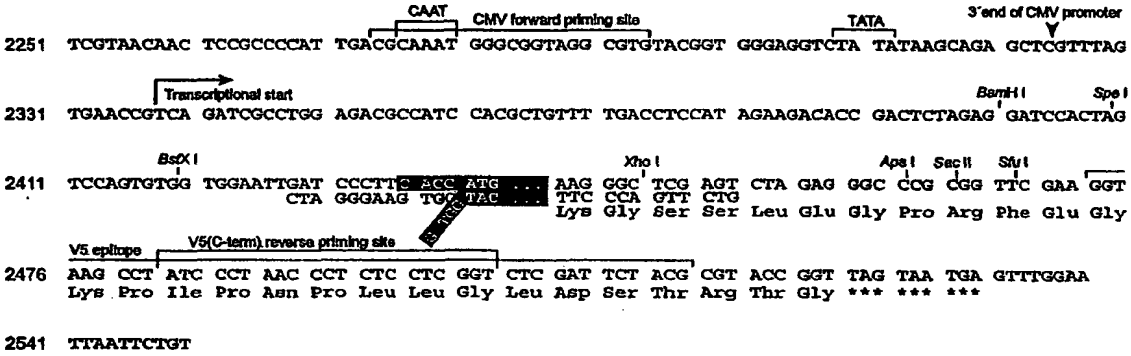
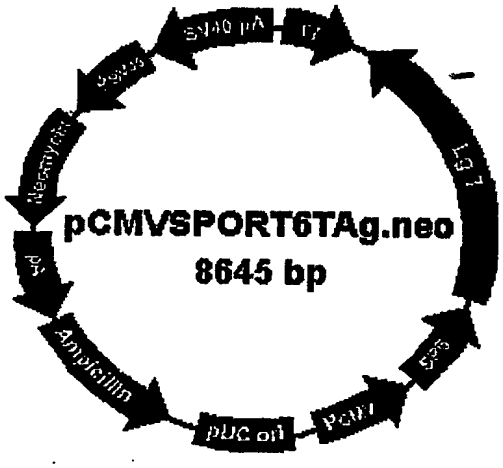

CAAT
CMV forward priming site
TATA
3'end of CMV promoter
Transcriptional start
BamH I
Spe I
BstX I
Xho I
Apa I
Sac II
Sfu I
V5 epitope
V5(C-term) reverse priming site
2251 TCGTAACAAC TCCGCCCCAT TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTCGTTTAG
2331 TGAACCGTCA GATCGCCTGG AGACGCCATC CACGCTGTTT TGACCTCCAT AGAAGACACC GACTCTAGAG GATCCACTAG
2411 TCCAGTGTGG TGGAATTGAT CCCTTC ACC ATG ... AAG GGC TCG AGT CTA GAG GGC CCG CGG TTC GAA GGT
CTA GGGAAG TGG TAC ... TTC CCA GTT CTG
Lys Gly Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly
5' TAC 3'
2476 AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTTGGAA
Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly *** *** ***
2541 TTAATTCTGT

FIG. 49


pCMVSPORT6TAg.neo
8645 bp
SV40 pA
f1
LacI
SP6
PCMV
pUC ori
Ampicillin
pA
Neomycin
SV40

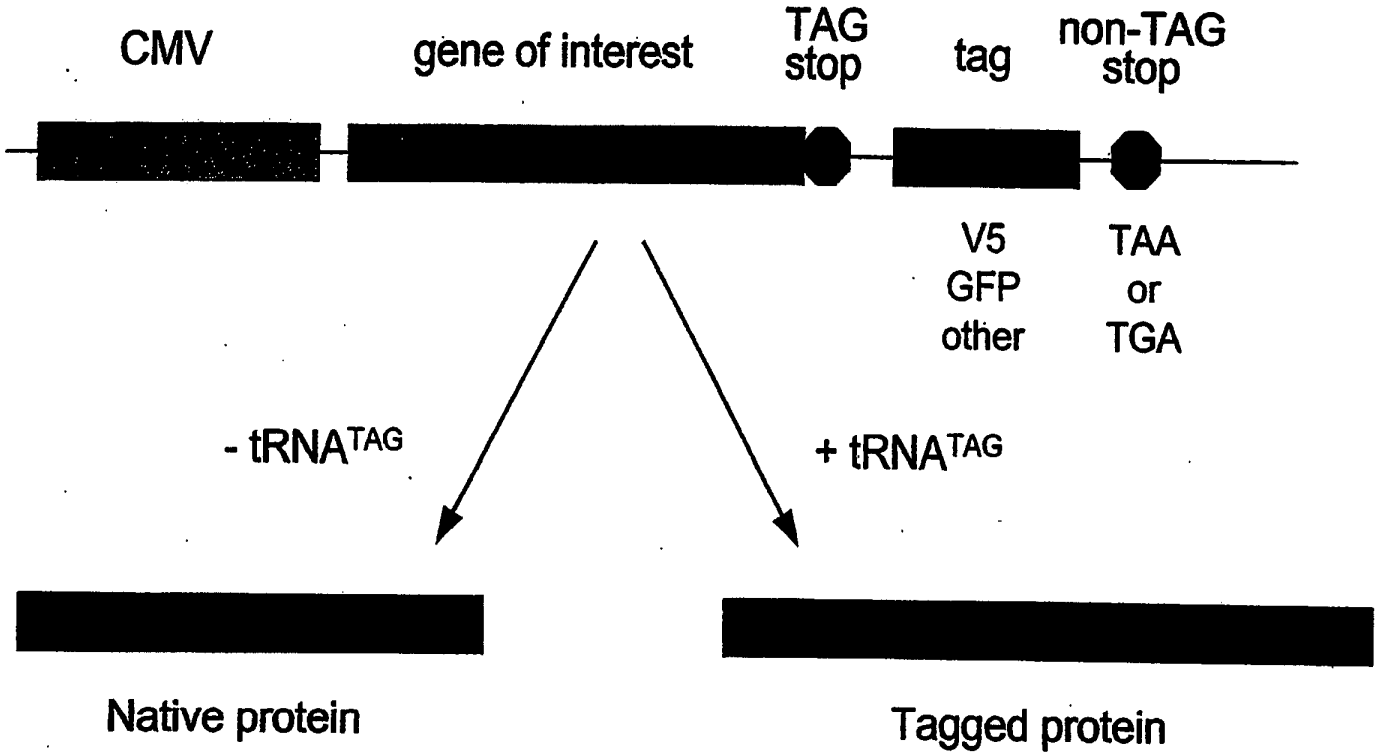

CMV
gene of interest
TAG stop
tag
non-TAG stop
V5 GFP other
TAA or TGA
- tRNA^TAG
+ tRNA^TAG
Native protein
Tagged protein

FIG. 50

FIG. 51A

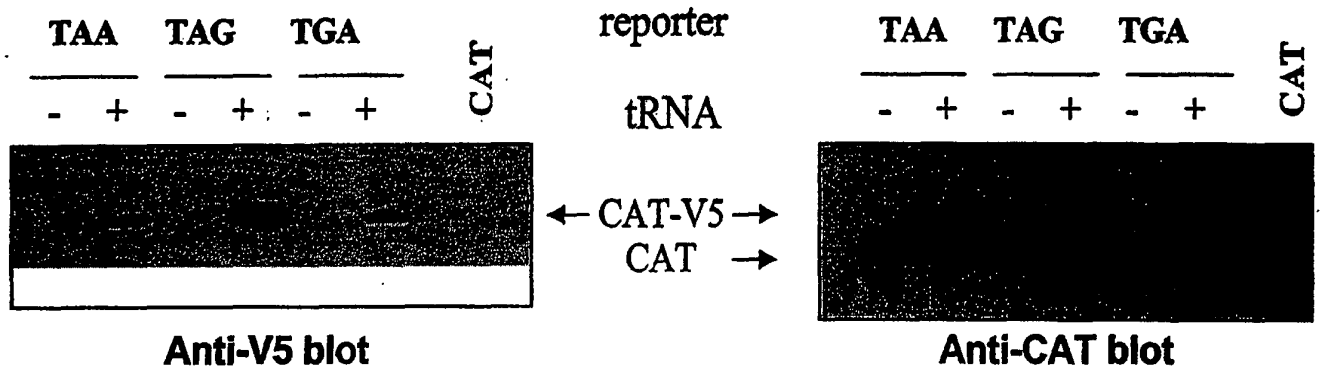
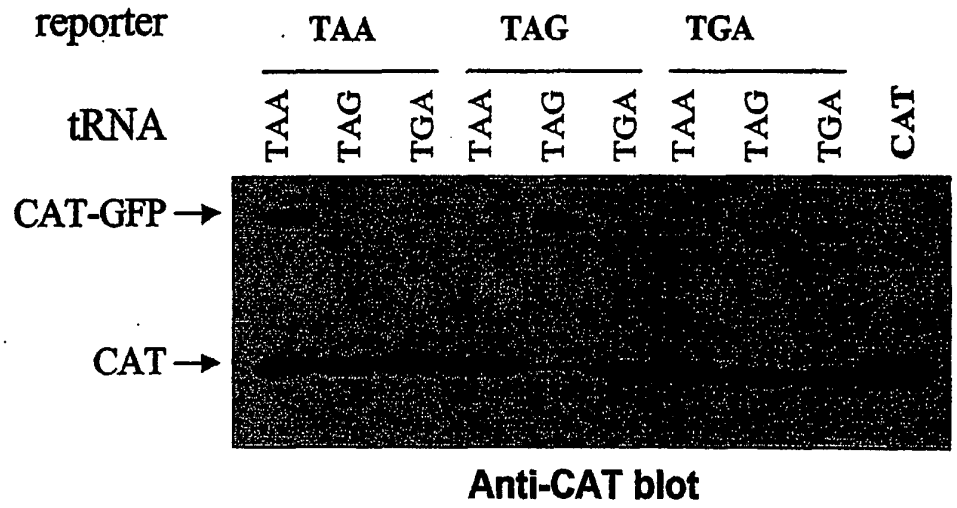


FIG. 51B



FIGS. 51 A-B

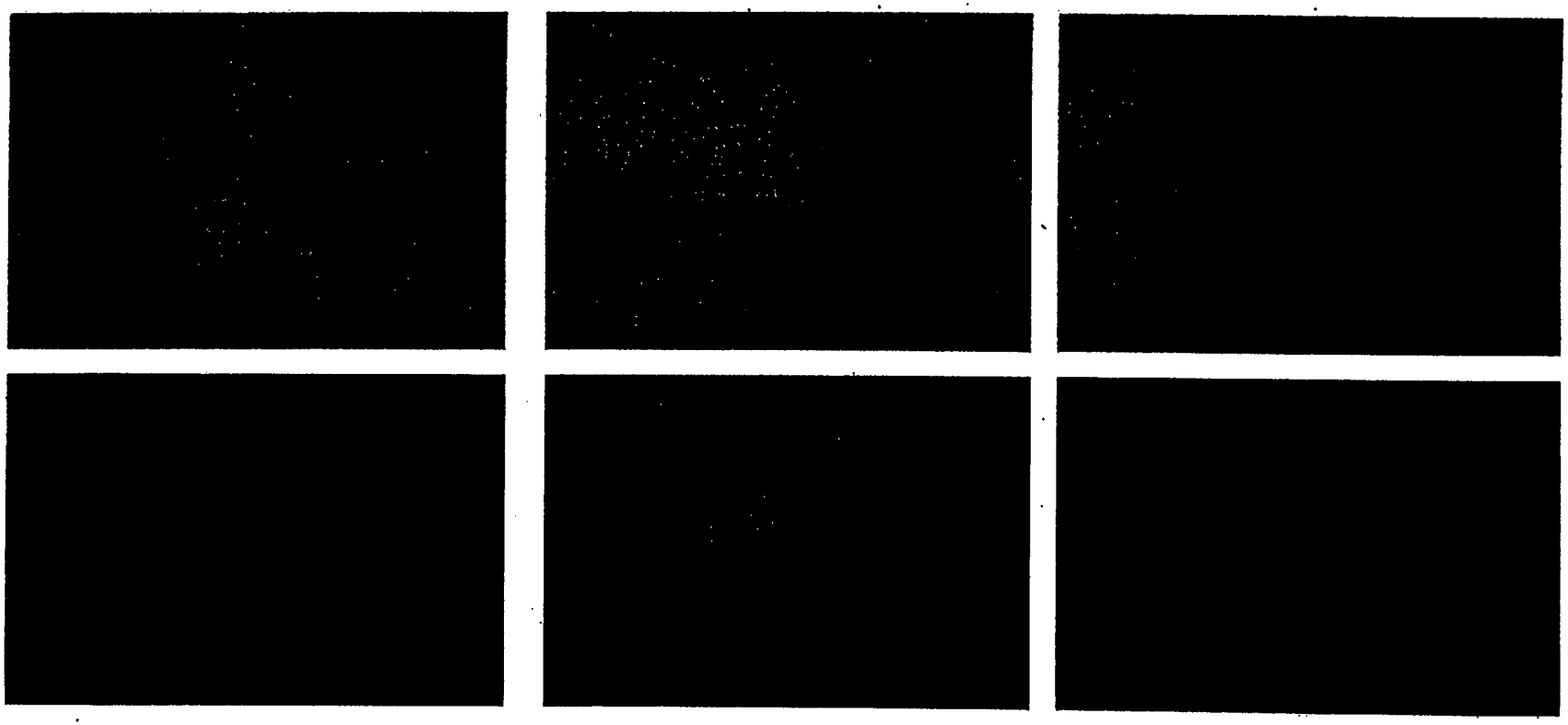


FIG. 52

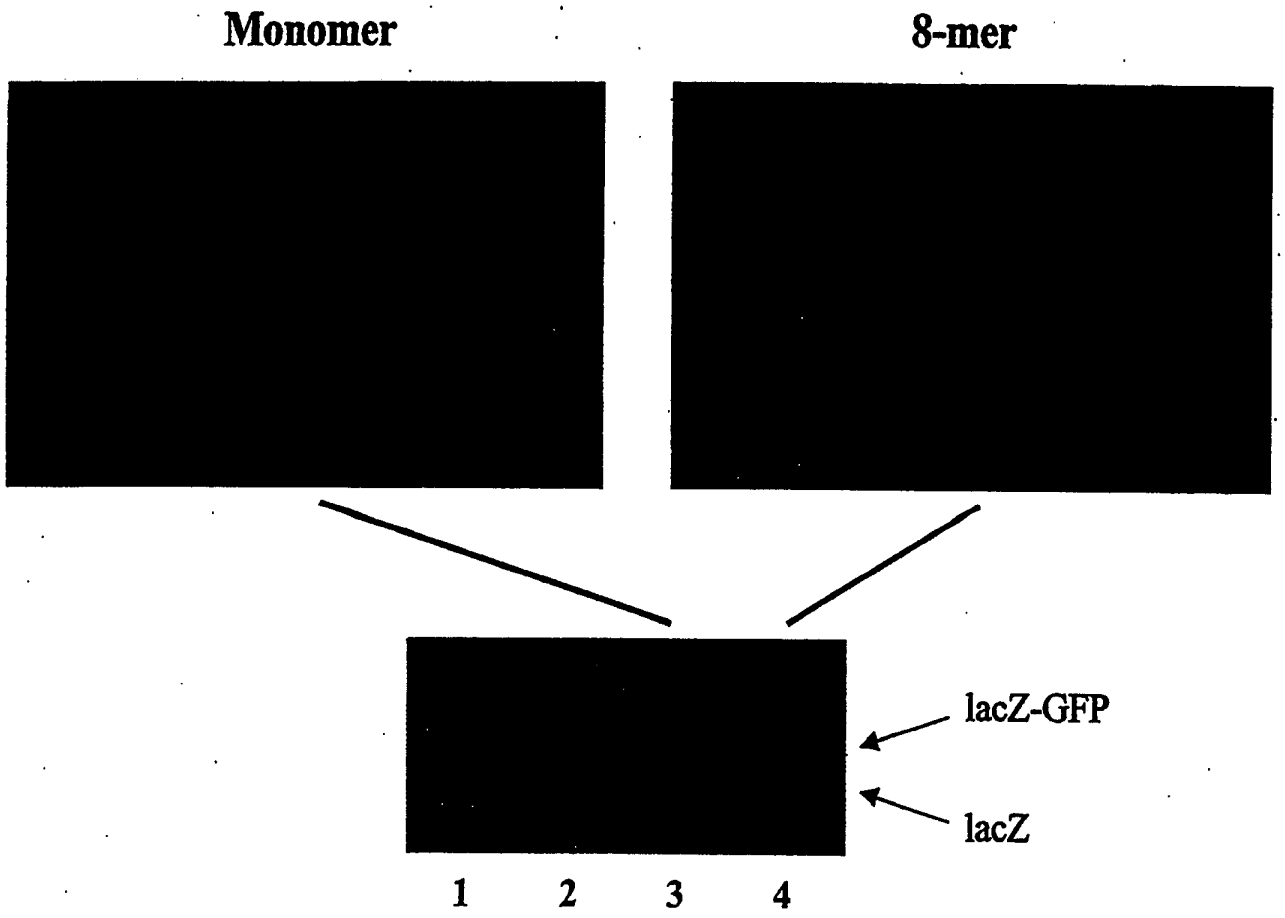
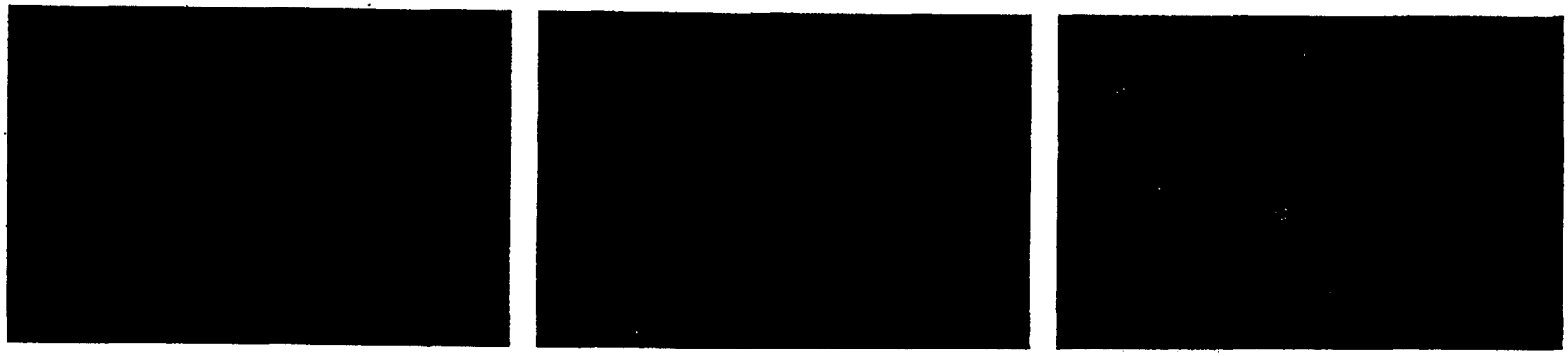
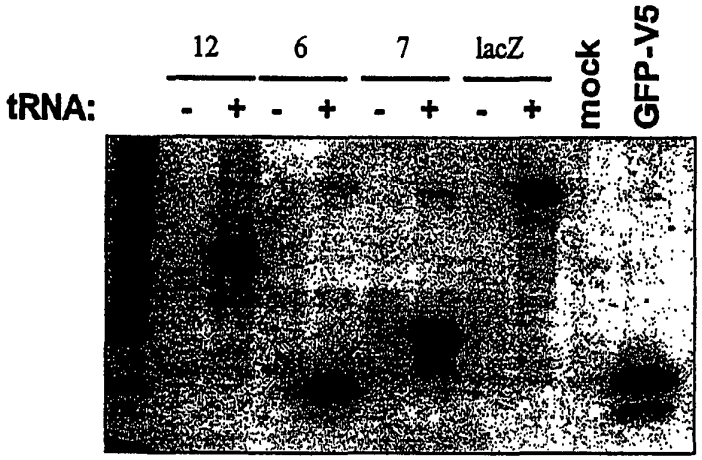


FIG. 53



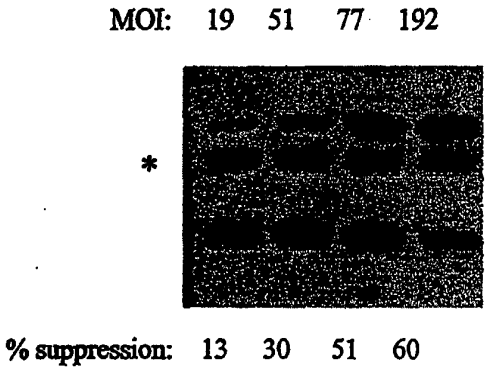
ORF6 (BC003357) ORF7 (BC000997) ORF12 (BC000141)



Anti-V5 blot

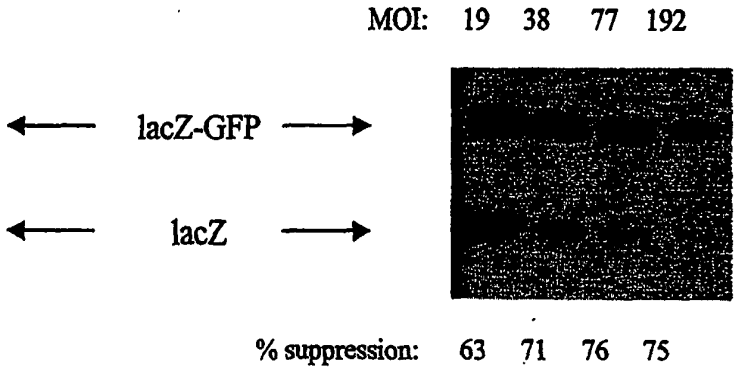
FIG. 54

FIG. 55A



STABLE GOI

FIG. 55B



TRANSIENT GOI

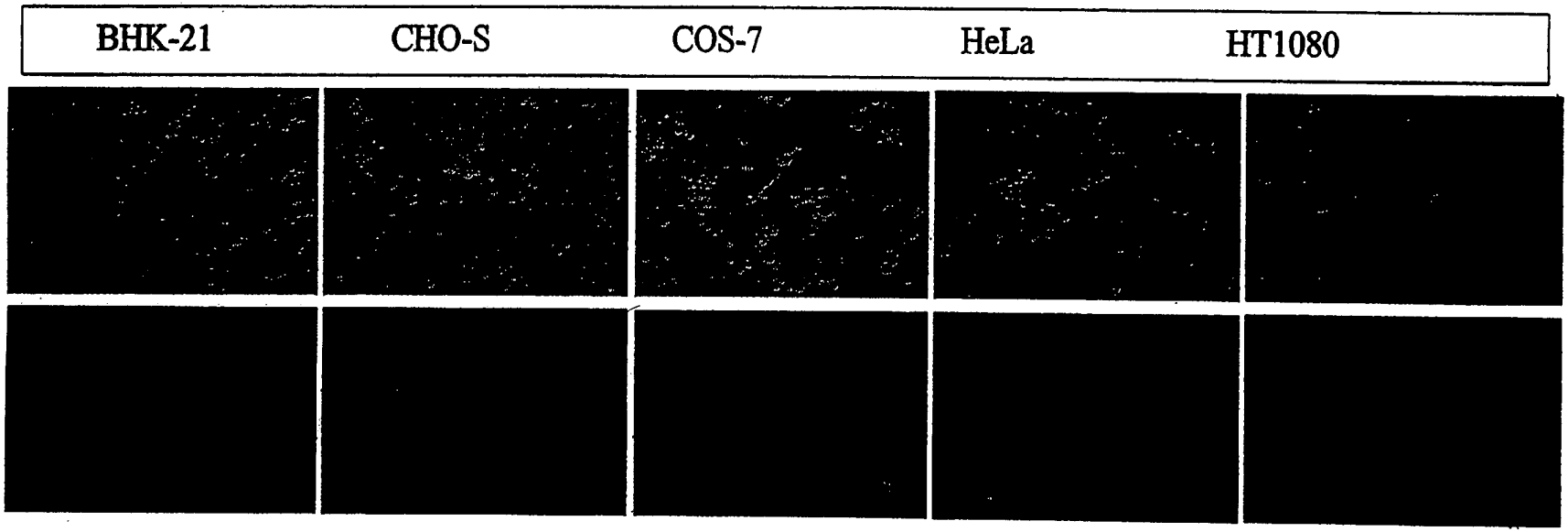


FIG. 56

FIG. 57

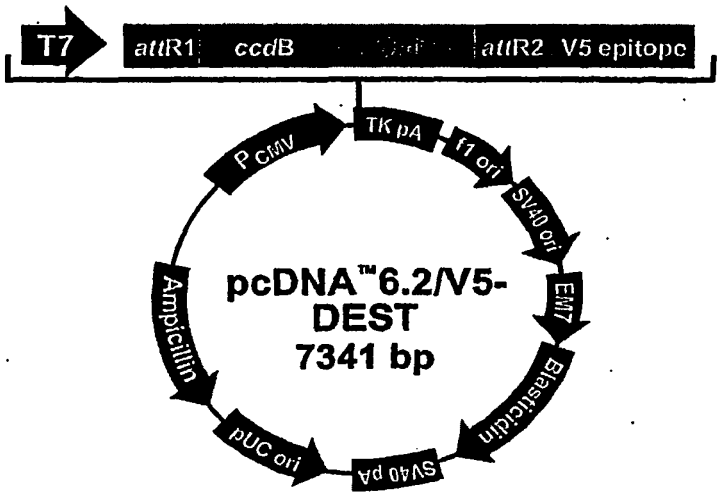

T7
attR1
ccdB
attR2
V5 epitope
P CMV
TK pA
f1 ori
SV40 ori
EM7
Blasticidin
SV40 pA
pUC ori
Ampicillin
pcDNA™6.2/V5-
DEST
7341 bp

FIG. 58

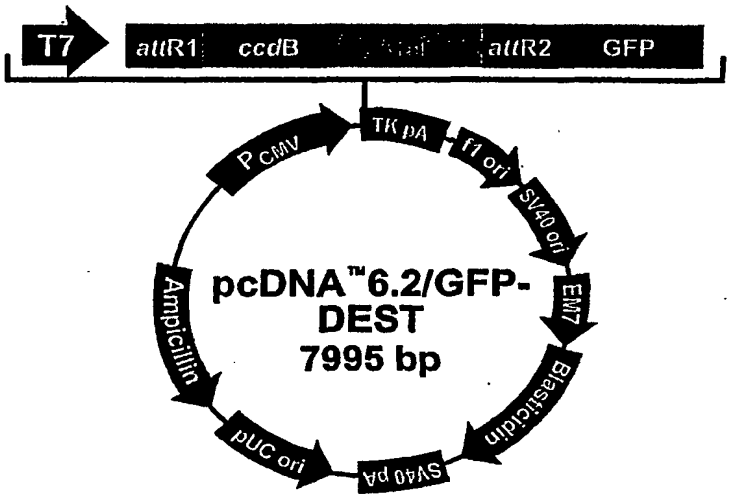

T7
attR1
ccdB
attR2
GFP
P CMV
TK pA
f1 ori
SV40 ori
EM7
Blasticidin
SV40 pA
pUC ori
Ampicillin
pcDNA™6.2/GFP-DEST
7995 bp

FIG. 59

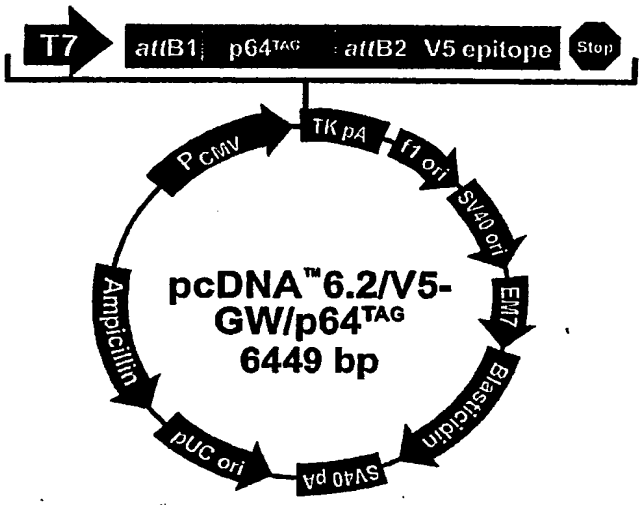

T7
attB1
p64TAG
attB2
V5 epitope
Stop
P CMV
TK pA
f1 ori
SV40 ori
EM7
Blasticidin
SV40 pA
pUC ori
Ampicillin
pcDNA™6.2/V5-GW/p64TAG
6449 bp

FIG. 60

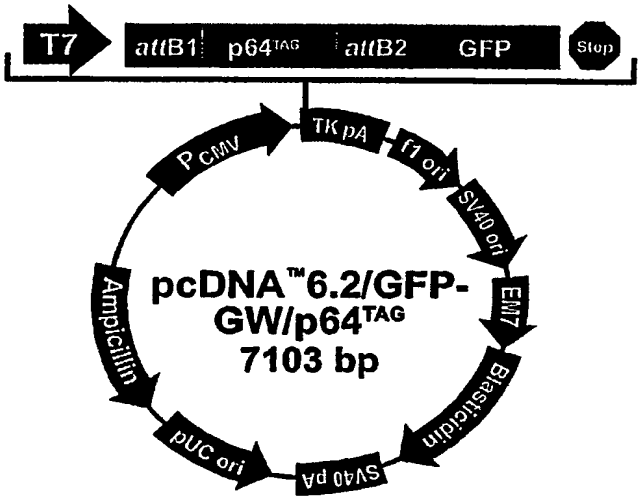
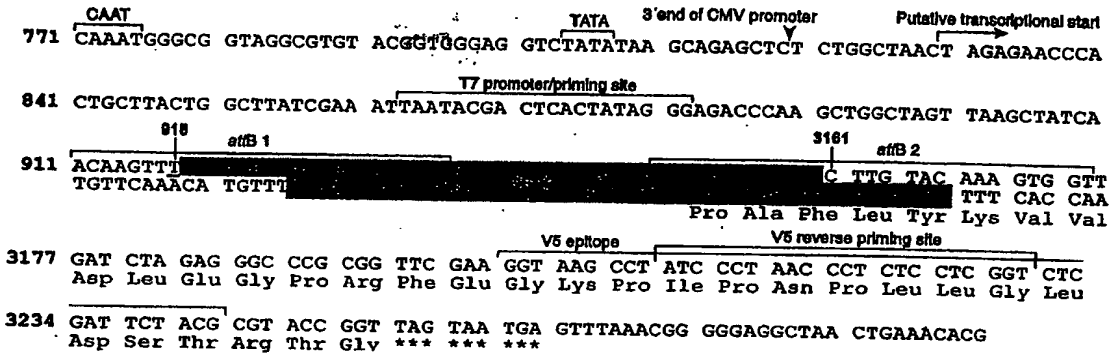

T7
attB1
p64TAG
attB2
GFP
Stop
TK pA
f1 ori
SV40 ori
EM7
Blasticidin
SV40 pA
pUC ori
Ampicillin
P CMV
pcDNA™6.2/GFP-GW/p64TAG
7103 bp

FIG. 61

A.


CAAT
TATA
3'end of CMV promoter
Putative transcriptional start
771 CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
T7 promoter/priming site
841 CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TAAGCTATCA
918
attB 1
3161
attB 2
911 ACAAGTTT
TGTTCAAACA TGTTT
C TTG TAC AAA GTG GTT
TTT CAC CAA
Pro Ala Phe Leu Tyr Lys Val Val
V5 epitope
V5 reverse priming site
3177 GAT CTA GAG GGC CCG CGG TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC
Asp Leu Glu Gly Pro Arg Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu
3234 GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTTAAACGG GGGAGGCTAA CTGAAACACG
Asp Ser Thr Arg Thr Gly *** *** ***

B.

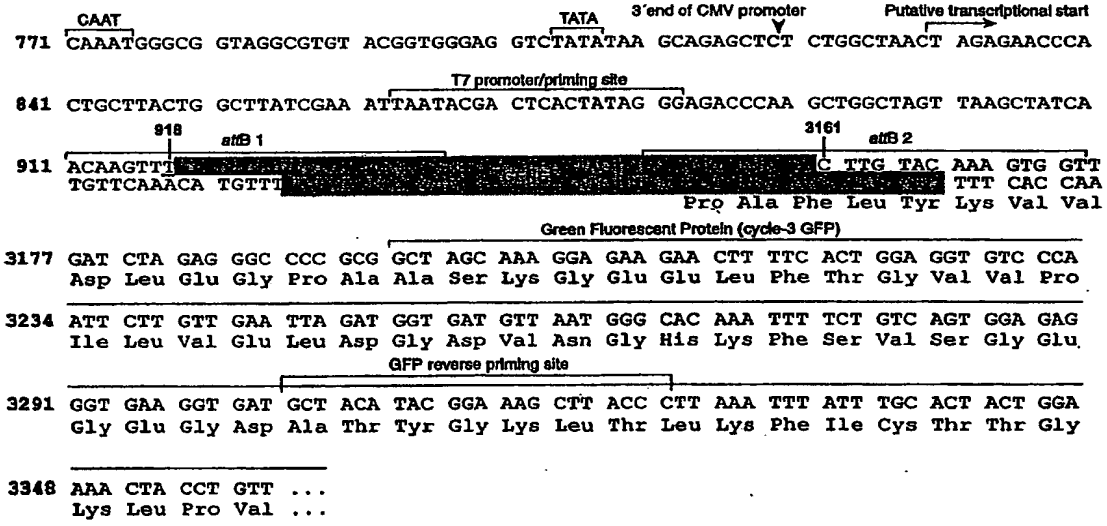
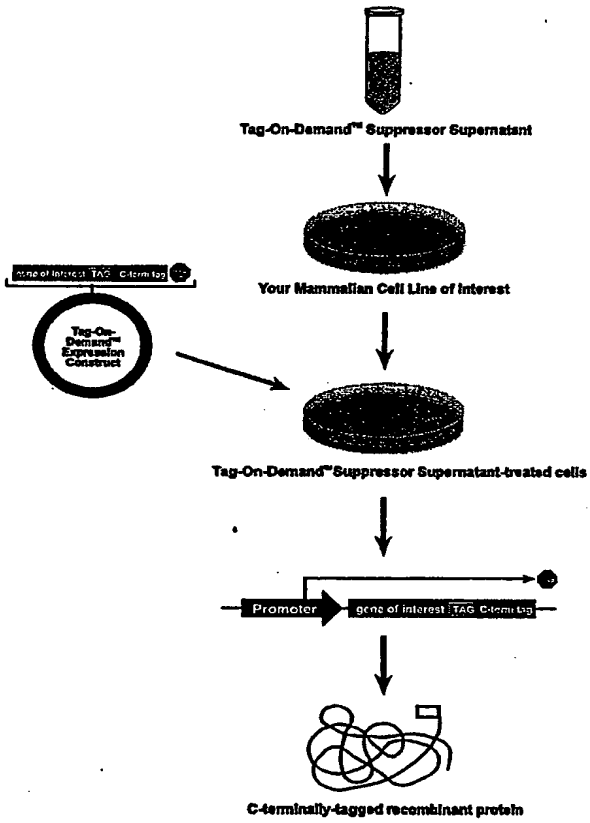

CAAT
TATA
3'end of CMV promoter
Putative transcriptional start
771 CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
T7 promoter/priming site
841 CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TAAGCTATCA
918
attB 1
3161
attB 2
911 ACAAGTTT
TGTTCAAACA TGTTT
C TTG TAC AAA GTG GTT
TTT CAC CAA
Pro Ala Phe Leu Tyr Lys Val Val
Green Fluorescent Protein (cycle-3 GFP)
3177 GAT CTA GAG GGC CCC GCG GCT AGC AAA GGA GAA GAA CTT TTC ACT GGA GGT GTC CCA
Asp Leu Glu Gly Pro Ala Ala Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
3234 ATT CTT GTT GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
GFP reverse priming site
3291 GGT GAA GGT GAT GCT ACA TAC GGA AAG CTT ACC CTT AAA TTT ATT TGC ACT ACT GGA
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly
3348 AAA CTA CCT GTT ...
Lys Leu Pro Val ...

FIG. 62



1. Add the Tag-On-Demand™ Suppressor Supernatant to cells.

2. Immediately transfect the Tag-On-Demand™ expression construct containing your gene of interest into treated cells.

3. Harvest cells after 24 hours and assay for C-terminally-tagged recombinant protein.

FIG. 63

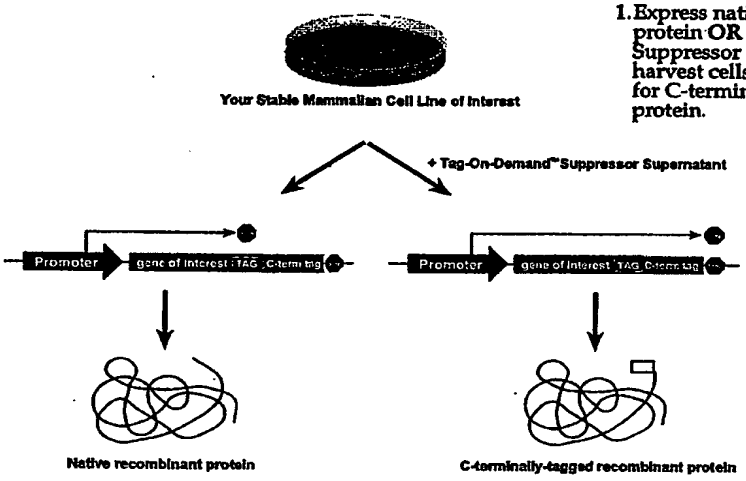
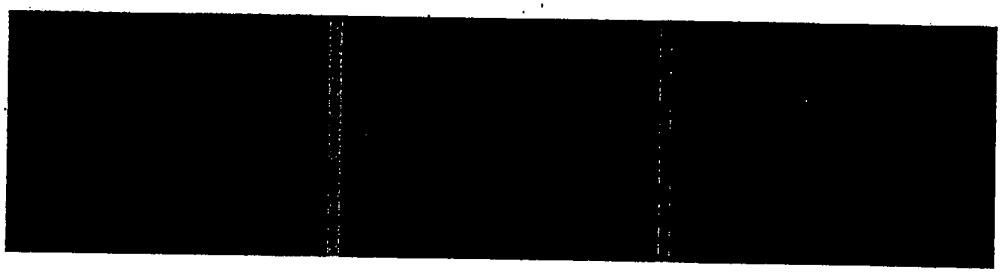

Your Stable Mammalian Cell Line of Interest
1. Express native recombinant protein OR add the Tag-On-Demand™ Suppressor Supernatant to cells, and harvest cells after 24-48 hours to assay for C-terminally-tagged recombinant protein.
+ Tag-On-Demand™ Suppressor Supernatant
Promoter
gene of interest
Promoter
Native recombinant protein
C-terminally-tagged recombinant protein

FIG. 64



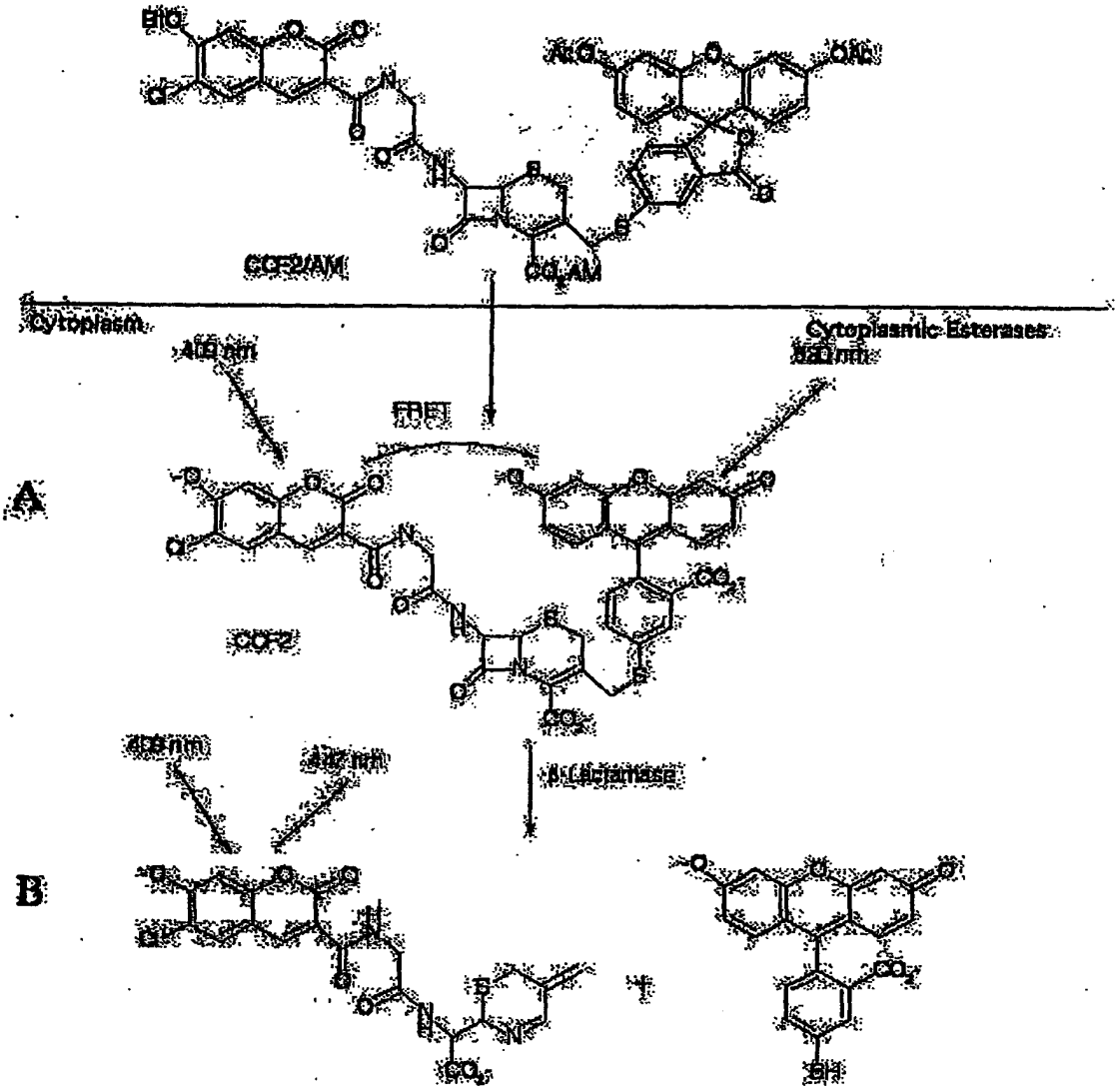

CCF2/AM
Cytoplasm
Cytoplasmic Esterases
FRET
A
CCF2
B
β-Lactamase

FIG. 65

Fig. 66

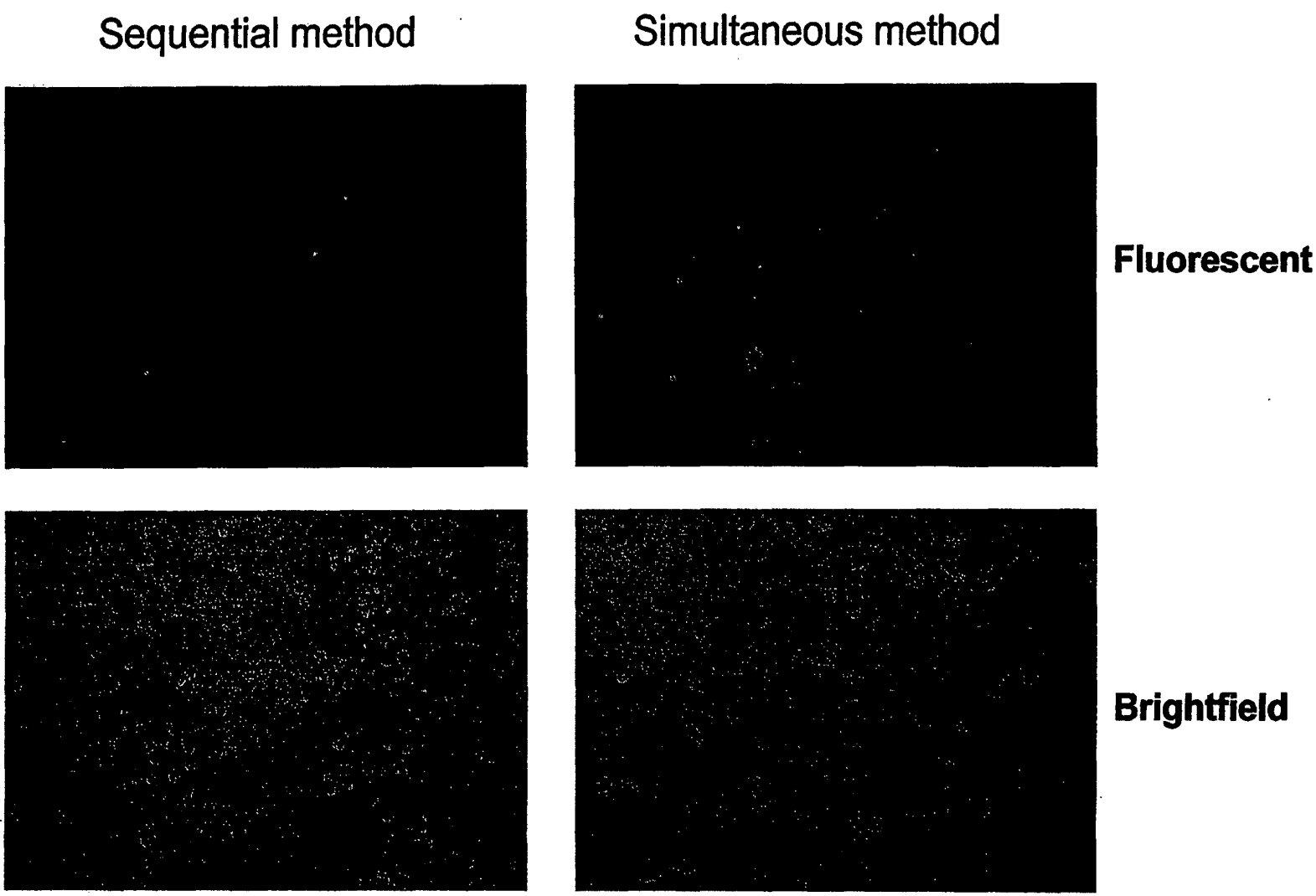
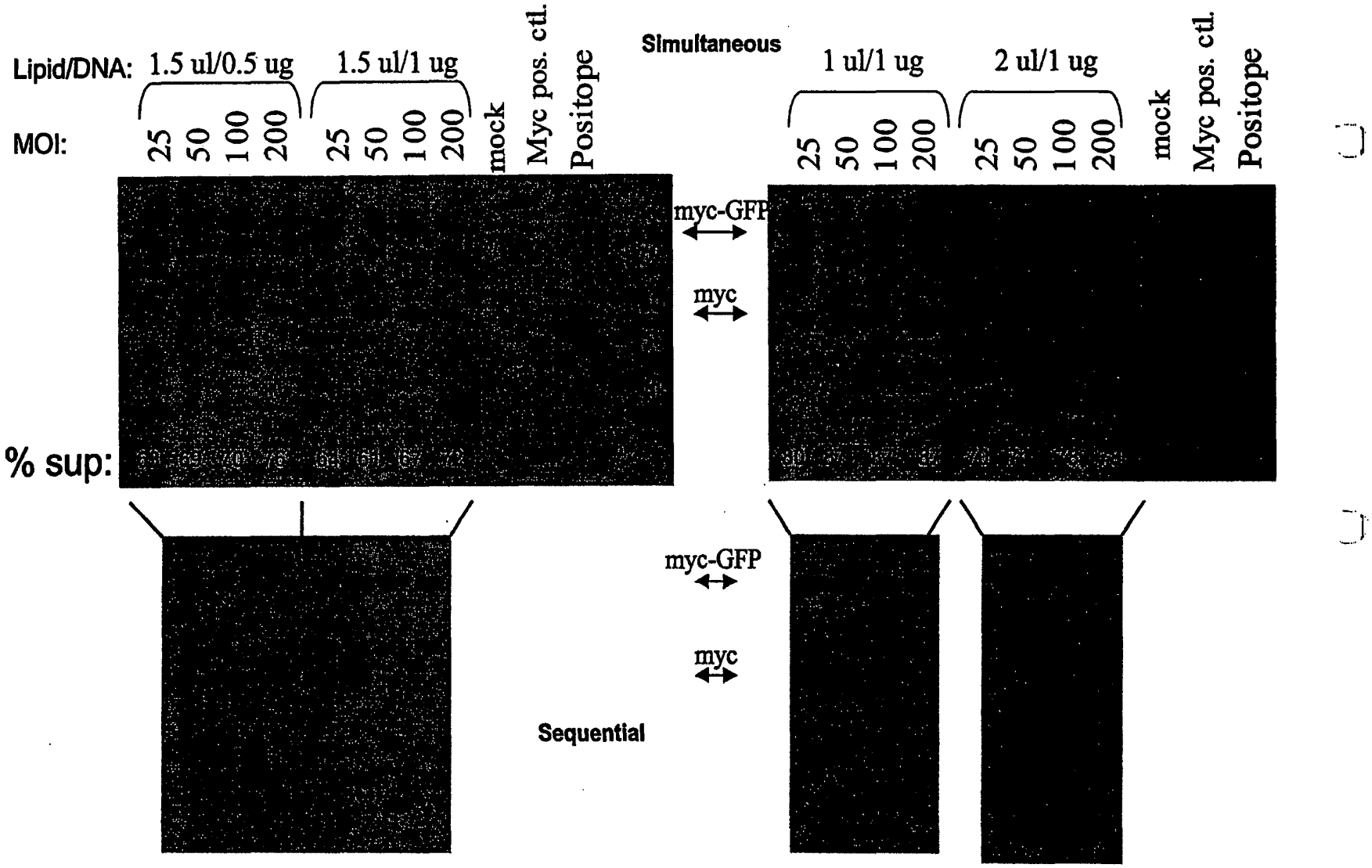

Sequential method
Simultaneous method
Fluorescent
Brightfield

FIG. 67


Simultaneous
Lipid/DNA:
1.5 ul/0.5 ug
1.5 ul/1 ug
1 ul/1 ug
2 ul/1 ug
MOI:
25
50
100
200
mock
Myc pos. ctl.
Positope
myc-GFP
myc
% sup:
Sequential

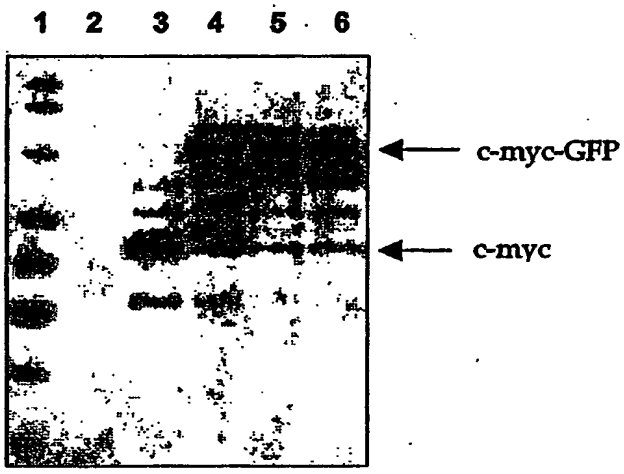


Fig. 6B

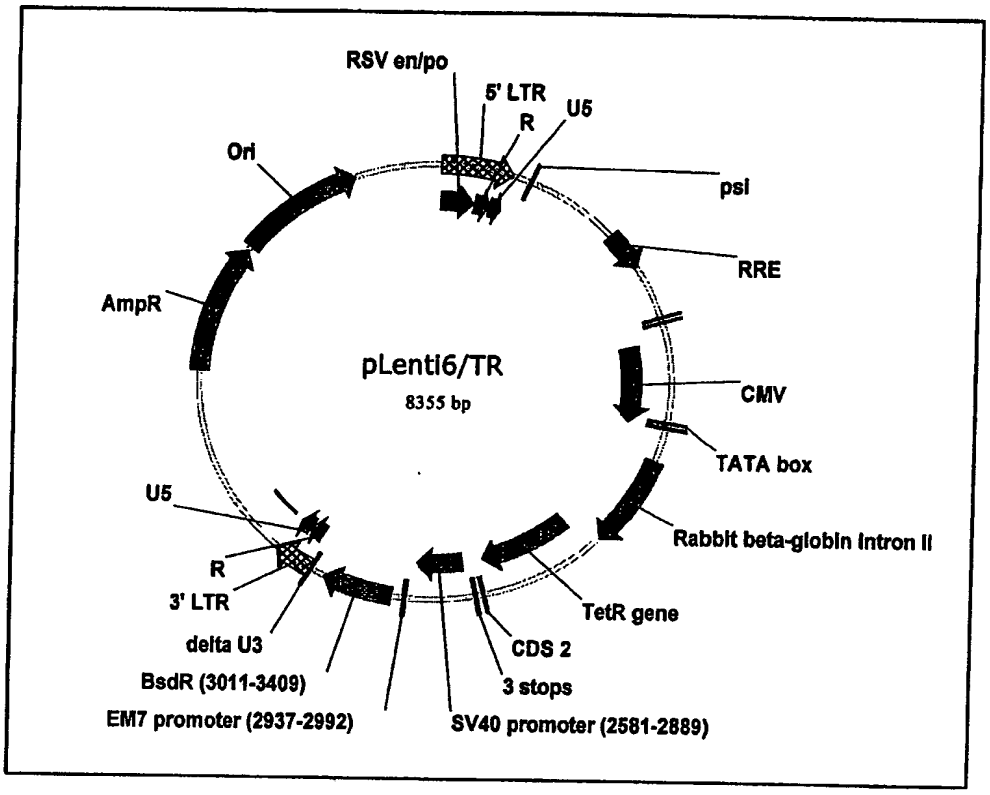

RSV en/po
5' LTR
R
U5
psi
RRE
CMV
TATA box
Rabbit beta-globin intron II
TetR gene
CDS 2
3 stops
SV40 promoter (2581-2889)
EM7 promoter (2937-2992)
BsdR (3011-3409)
delta U3
3' LTR
R
U5
AmpR
Ori
pLenti6/TR
8355 bp

FIG. 69

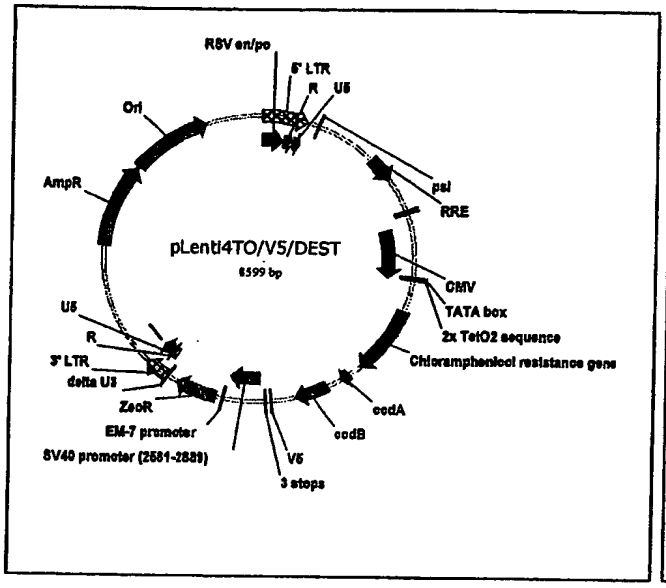


FIG. 70A

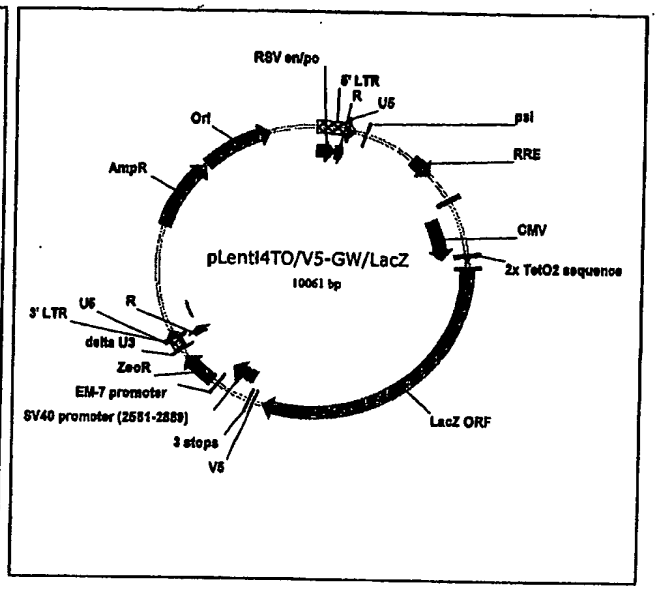


FIG. 70B

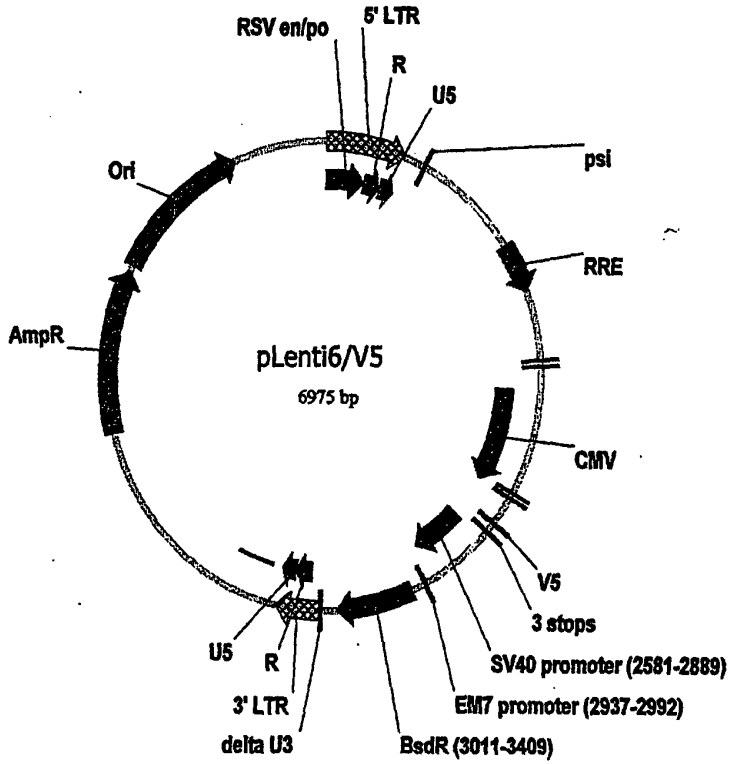

RSV en/po
5' LTR
R
U5
psi
RRE
CMV
V5
3 stops
SV40 promoter (2581-2889)
EM7 promoter (2937-2992)
BsdR (3011-3409)
delta U3
3' LTR
R
U5
AmpR
Ori
pLenti6/V5
6975 bp

FIG. 71

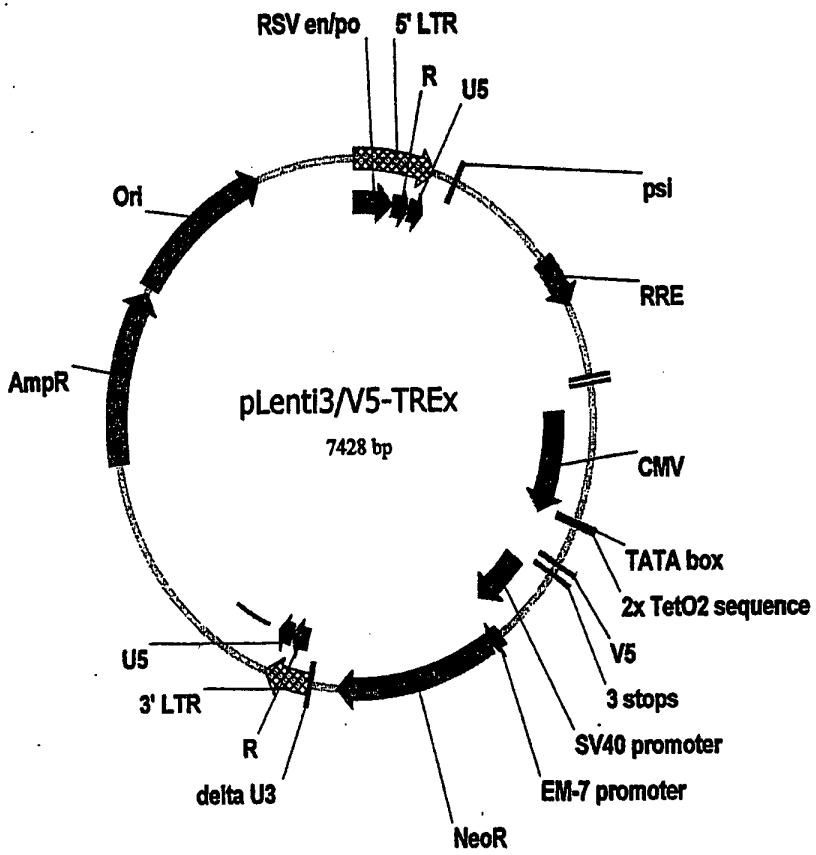

RSV en/po
5' LTR
R
U5
psi
RRE
CMV
TATA box
2x TetO2 sequence
V5
3 stops
SV40 promoter
EM-7 promoter
NeoR
delta U3
R
3' LTR
U5
Ori
AmpR
pLenti3/V5-TREx
7428 bp

FIG. 72

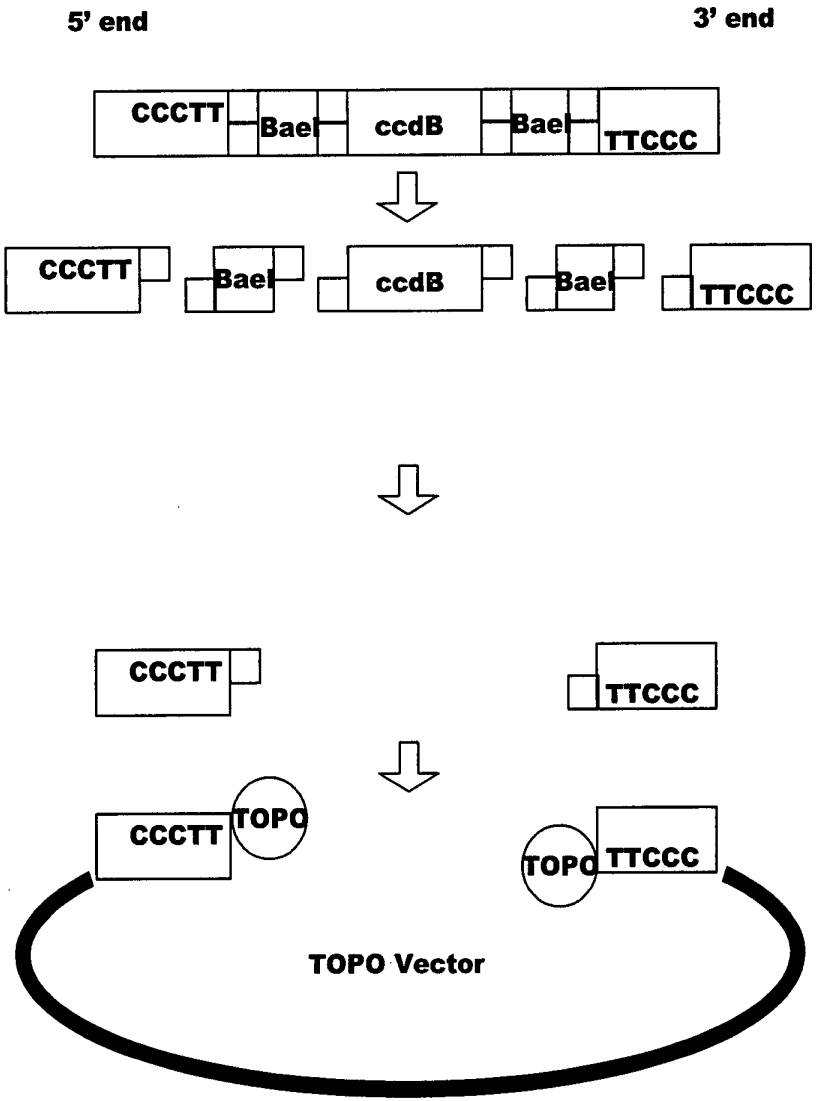

5' end
3' end
CCCTT
BaeI
ccdB
BaeI
TTCCC
CCCTT
BaeI
ccdB
BaeI
TTCCC
CCCTT
TTCCC
CCCTT
TOPO
TOPO
TTCCC
TOPO Vector

FIG. 73